UTILITY APPLICATION

BY

Paul S. Keim

And

Kristen Zinnamon

FOR

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ON

DNA FINGERPRINTING FOR CANNABIS SATIVA (MARIJUANA) USING SHORT TANDEM REPEAT (STR) MARKERS

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CLAIM TO DOMESTIC PRIORITY

[0001] This application claims benefit of priority to US Provisional application Serial No. 60/397,179, entitled "DNA Fingerprinting For *Cannabis sativa* (Marijuana) Using Short Tandem Repeat (STR) Markers" filed July 19, 2002, by Paul S. Keim et al., and is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0002] This invention concerns the molecular analysis of *Cannabis sativa* L. (marijuana) and more specifically provides primer cocktails for multiplex analysis of DNA from purported *Cannabis sativa* L. samples to allow forensic identification and tracking of a leaf sample to its plant source.

BACKGROUND

[0003] *Cannabis sativa* L. is one of the oldest crops known to man (Siniscalco Gigliano 2001). Despite its long historical relationship with human civilization, still relatively little is known about the genetic composition of this plant. However, recently many studies have tried to examine the molecular characteristics of *Cannabis* in order to distinguish hemp (fiber) varieties from marijuana (drug) varieties (Gilmore et al. 2003).

[0004] The historical and intimate association between *Cannabis sativa* L. (marijuana) and man has no doubt contributed to this plant's many varieties and uses [1,2]. It is commonly believed that humans introduced *C. sativa* to the Americas in 1545; but before its worldwide introduction, it likely originated and was native to central Asia [3,4]. From even the earliest accounts, man has utilized virtually all parts of the plant for a multitude of purposes, the two most common uses being harvesting the plant for its fiber and drug qualities [5]. The flowers and leaves of the plant are harvested for the chemical resin, delta-9-tetrahydrocannabinol (THC), which when ingested, produces the psychoactive effects that humans experience [6].

[0005] A common problem for law enforcement agencies is the correct identification and suppression of illegal growing operations. The forensic community has made significant progress in developing molecular identification techniques for *Cannabis* [7-11]. Virtually all of these experiments have focused on molecular identification methods which exclusively amplify *Cannabis* DNA, enabling forensic investigators to move away from conventional chemical identification tests such as GC-MS, HPLC and histological microscopy. Despite these advances, tests that are capable of individualizing marijuana plants and discriminating between varieties were not available, until recently [12,13]. These kinds of tests are necessary to facilitate the identification and suppression of growing operations by forensic investigators.

[0006] Both Gilmore [12] and Hsieh [13] have investigated the potential utility of short tandem repeat (STR) markers for distinguishing and individualizing *Cannabis* plants. Short tandem repeats (STRs), simple sequence repeats (SSRs), or microsatellites all describe a single type of DNA profiling technology that is useful for providing genetic information about individuals within and among populations. STR genetic markers selectively amplify hypervariable regions of DNA and, when run on gels, generate fluorescent banding patterns that can be used as unique genetic identifiers. Each STR marker is made up of a single DNA sequence, no more than six base pairs long, that is repeated in tandem and individual loci have length polymorphisms in the repeat array [14]. STR markers are useful in forensic investigations because they are polymerase chain reaction (PCR) based and are capable of amplifying small amounts of fairly degraded DNA, which is commonly the condition of biological samples from crime scenes [14]. Additionally, STR markers are desirable because they are a co-dominant marker system and they provide information about the heterozygosity of individual plants.

[0007] Methods and means for reliable and fast genetic analysis of STR markers in *Cannabis sativa* L. have been sought. These analyses would identify purported marijuana samples and would provide a useful forensic tool for linking the source of sample to its plant of origin.

[0008] It is an object of this invention to provide methods and means for STR typing in *Cannabis* to aid forensic investigators in: (i) linking personal possessions of marijuana to plants at the person's residence, (ii) identifying clonally propagated plants as having matching genotypic profiles, and (iii) tracking the distribution patterns of clonally propagated plants within residential areas.

SUMMARY

[0009] The present invention discloses methods and means for detecting and identifying *Cannabis sativa* L. species by short tandem repeat (STR) analysis multiplex genotyping system of STR identified within the genome of *Cannabis sativa* L. STR in the *Cannabis sativa* L. genome are amplified using labeled primers in multiplexed PCRs and electrophoretically separated on polyacrylamide gels for analysis.

[0010] STR loci located throughout the *Cannabis sativa* L. genome have been identified. Isolated nucleic acids having the sequence of STR identified in *Cannabis sativa* L. are presented. In an important aspect of the present invention nucleic acids comprising at least 12, 15, 18 or total consecutive nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; SEQ ID NO: 4; SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; SEQ ID NO: 10; SEQ ID NO: 11; SEQ ID NO: 12; SEQ ID NO: 13; SEQ ID NO: 14; SEQ ID NO: 15; SEQ ID NO: 16; SEQ ID NO: 17; SEQ ID NO: 18; SEQ ID NO: 19; SEQ ID 20; SEQ ID 21; SEQ ID 22; SEQ ID 23; SEQ ID 24; SEQ ID 25; SEQ ID 26; SEQ ID 27; SEQ ID 28; and sequences complementary thereto are presented.

[0011] In certain preferred embodiments of the invention, these nucleic acids are immobilized on a solid surface and are useful, for example, in the detection of a *Cannabis sativa* L. sample in an assay employing probes, including, but not limited to, a nano-detection device.

[0012] In another important aspect of the invention, primer pairs comprising a forward and a reverse primer are presented for amplification of STR located in DNA from a *Cannabis sativa* L. species. Primer pairs suitable for PCR amplification of STR, by multiplex, may be selected from the group consisting of SEQ ID NO: 1 and 2; SEQ ID

NO: 3 and 4; SEQ ID NO: 5 and 6; SEQ ID NO: 7 and 8; SEQ ID NO: 9 and 10; SEQ ID NO: 11 and 12; SEQ ID NO: 13 and 14; SEQ ID NO: 15 and 16; and SEQ ID NO: 17 and 18; SEQ ID NO: 19 and 20; SEQ ID NO: 21 and 22; SEQ ID NO: 23 and 24; SEQ ID NO: 25 and 26; and SEQ ID NO: 27 and 28.

[0013] Combinations of the isolated nucleic acids or primer pairs described herein as "cocktails" are provided for amplification of the STR markers by multiplex. Certain preferred primer pairs have, in addition, an observable group whereby amplified product may be detected. Such groups may be, for example, a fluorescent group or a radioactive group.

[0014] In another important aspect of the invention, a method for detecting a *Cannabis sativa* L. species in a sample from a plant, preferably a leaf or flower sample, is presented. The method comprises the steps of:

i. obtaining DNA from the sample,

ii. amplifying a STR marker loci in said DNA with a multiplex cocktail selected from the group of primer pairs to form amplification products of various sizes and labels; and

iii. separating amplification products by size and primer label;

iv. scoring the results of said separation

v. comparing said scored results to results of analysis of DNA from a known species.

[0015] In yet another important aspect of the invention methods for linking a marijuana sample to a plant source are presented. The method comprises the steps of:

i. determining the identity of DNA in said sample by the present method

ii. determining the identity of DNA in a sample from a plant by the present method; and

iii. comparing the identities of both samples to determine similarities.

[0016] In another important aspect of the invention, multiplex methods are presented for observing polymorphisms at STR loci in DNA from more than one *Cannabis sativa* L. species to resolve unique genotypes between the species and to allow linking of the sample to its plant of origin. These multiplex methods provide a

convenient and rapid method for genetic discrimination in *Cannabis sativa* L. and, for forensic purposes, provides information necessary to track the source of a purported marijuana sample. Cocktails provided herein are preferably used for amplifying STR in the multiplex methods.

[0017] In yet another important aspect of the invention, kits are herein provided for use with commercially available PCR instruments to detect a strain of *Cannabis sativa* L. species. The kits comprise one or more primer pairs suitable for amplifying STR in DNA in a sample of said species by PCR. Preferably the kits comprise primer pairs having SEQ ID NOS: 1-28. Most preferably kits are provided for multiplexing DNA in a sample. These kits comprise primer pair sets, i.e., cocktails, selected from the group of primer pairs.

[0018] The kits may further comprise nucleic acids, enzymes, tag polymerase, for example, salts and buffers suitable for causing amplification by PCR, by multiplex. The kits also comprise preferably a positive control. In certain preferred embodiments of the kit the primers comprise a label whereby amplified STR may be detected. In other preferred embodiments of the kit, labeled nucleic acids are provided. Observable labels are preferably fluorescent molecules or radionucleotides. The kits may also comprise suitable containers and bottles for housing these reagents and or convenient use.

DETAILS

[0019] Multiplex methods are presented for rapid genotyping of *Cannabis sativa* L. STR markers described herein provide discriminatory power that enhances the ability of present methods to determine rapidly molecular relationships of *Cannabis sativa* L. samples. A *C. sativa* STR database has been generated by multiplexing 295 samples and eight STR markers. This database illustrates that STR genetic markers in *C. sativa* are both hypervariable and capable of discriminating among individual plants.

[0020] This multiplex typing system is a PCR-based method for genotyping *Cannabis sativa* L. using eight STR loci identified in the present invention. This PCR-based typing system has advantages not present in other PCR-systems: rapid turnaround, amplification with crudely isolated or minute amounts, of DNA. The rapid typing system using eight STR loci has been used to analyze a collection of a 295 samples to detect

genotypic differences between individual *C. sativa* plants. Over 90% of the samples had unique multilocus genotypic profiles and some of the samples with matching profiles were known to be duplicate samples. Although the heterozygosity values detected within this system are fairly low compared to other studies of STRs in plants [12,18], this may be indicative of the selective breeding practices within drug varieties of *C. sativa* plants. It is known that certain drug qualities such as THC content are selectively bred for within this plant [24] and therefore, this system may be detecting some of these highly inbred genotypes. Additional markers, [12,13] would increase the observed heterozygosity values and enhance the power of an STR profiling system for *C. sativa*.

[0021] Tri- and tetranucleotide repeat motifs were isolated for their ease of scoring and preferential use in the forensic community [25,26]. Additionally, the observed allele size range (103–364bp) for these markers allows for rapid data collection and accurate scoring due to these smaller fragment sizes [26]. The present system detected 63 alleles. The method of detection may be applied to discover more alleles in other plant samples, including fiber varieties.

[0022] The following definitions are used herein:

[0023] "Polymerase chain reaction" or "PCR" is a technique in which cycles of denaturation, annealing with primer, and extension with DNA polymerase are used to amplify the number of copies of a target DNA sequence by approximately 106 times or more. The polymerase chain reaction process for amplifying nucleic acid is disclosed in US Patent Nos. 4,683,195 and 4,683,202, which are incorporated herein by reference.

[0024] "Primer" is a single-stranded oligonucleotide or DNA fragment which hybridizes with a DNA strand of a locus in such a manner that the 3' terminus of the primer may act as a site of polymerization using a DNA polymerase enzyme.

[0025] "Primer pair" is two primers including, primer 1 that hybridizes to a single strand at one end of the DNA sequence to be amplified and primer 2 that hybridizes with the other end on the complementary strand of the DNA sequence to be amplified.

[0026] "Primer site" the area of the target DNA to which a primer hybridizes.

[0027] "Multiplexing" is a capability to perform simultaneous, multiple determinations in a single assay process and a process to implement such a capability in a process is a "multiplexed assay." Systems containing several loci are called *multiplex* systems described, for example, in US Patent No. 6,479,235 to Schumm, et al., US Patent No. 6,270,973 to Lewis, et al. and 6,449,562 to Chandler, et al.

[0028] "Cocktail" is a mixture of primer pairs selected to amplify one or more STR loci in a multiplex system.

[0029] Isolated nucleic acid" is a nucleic acid which may or may not be identical to that of a naturally occurring nucleic acid. When "isolated nucleic acid" is used to describe a primer, the nucleic acid is not identical to the structure of a naturally occurring nucleic acid spanning at least the length of a gene. The primers herein have been designed to bind to sequences flanking STR loci in *Cannabis sativa* species. It is to be understood that primer sequences containing insertions or deletions in these disclosed sequences that do not impair the binding of the primers to these flanking sequences are also intended to be incorporated into the present invention.

Forensic Utility of STR Markers

[0030] Databases compiled by the present system will be used for drug trafficking and intelligence purposes and to track distribution patterns and growing operations. Additionally, databases are going to be necessary for gaining court acceptance of *Cannabis* DNA fingerprinting systems [12,28].

[0031] Recently, the forensic community has expressed considerable interest in non-human DNAfingerprinting methods for assisting in criminal investigations [27,28]. With the present STR system, forensic investigators will be able to generate genetic profiles of individual *C. sativa* plants and compare them to databases [12,28] or to suspected clonally propagated plants to determine if the profiles match. The identification of clonal growing operations and tracking distribution patterns of individual *Cannabis* plants has the greatest immediate potential for this system. The ability to generate matching genotypic profiles from plants confiscated from independent locations within the same residential area would support the hypothesis that the plants were coming from the same clonal growing operation.

Development of STR Markers

[0032] Of the seven arbitrary repeat motifs that were screened in this protocol, only three (AGC, AAAG, CCT) yielded sequences with sufficient flanking regions for primer development. Over two hundred individual positive clones were sequenced to find a total of 33 sequences that contained repeat motifs with at least five repeating units and sufficient flanking sequence on either side of the repeat. Of the 15 markers that were identified as polymorphic, only eight amplified consistently and were easy to score, with minimal stutter problems (Table 2).

Locus Name Dye Label[a]	Repeat Motifs*	Aplicon Size Range (bp)	Number of Alleles	Multiplex Mix #
AAAG1 HEX	(AAAG)6	103-135	16	1
ACT1 FAM	(ACT)6	218-224	3	1
AGC8 NED & FAM	(AGC)5	264-279	6	1
AGC9 HEX	(AGC)9	317-335	7	1
AGC1 FAM	(AGC)10	128-164	10	2
AAAG5 NED	(AAAG)5	188-200	4	2
AAAG7 FAM	(AAAG)6	242-266	7	3
AAAG10 FAM	(AAAG)5	352-364	4	3
AGC6 HEX	(AGC)6	200 & 221	2	3
AGC10 NED	(AGC)43	273-327	15	3

These primer sequences have herein been assigned SEQ ID NO: as follows:

SEQ ID NO	Marker Name	
SEQ ID NO: 1	AAAG1	Forward primer

SEQ ID NO: 2	AAAG1	Reverse primer
SEQ ID NO: 3	AAAG5	Forward primer
SEQ ID NO: 4	AAAG5	Reverse primer
SEQ ID NO: 5	AAAG6	Forward primer
SEQ ID NO: 6	AAAG6	Reverse primer
SEQ ID NO: 7	AAAG7	Forward primer
SEQ ID NO: 8	AAAG7	Reverse primer
SEQ ID NO: 9	AAAG10	Forward primer
SEQ ID NO: 10	AAAG10	Reverse primer
SEQ ID NO: 11	AAAG11	Forward primer
SEQ ID NO: 12	AAAG11	Reverse primer
SEQ ID NO: 13	AGC1	Forward primer
SEQ ID NO: 14	AGC1	Reverse primer
SEQ ID NO: 15	AGC3	Forward primer
SEQ ID NO: 16	AGC3	Reverse primer
SEQ ID NO: 17	AGC6	Forward primer
SEQ ID NO: 18	AGC6	Reverse primer
SEQ ID NO: 19	AGC8	Forward primer
SEQ ID NO: 20	AGC8	Reverse primer
SEQ ID NO: 21	AGC9	Reverse primer
SEQ ID NO: 22	AGC9	Reverse primer
SEQ ID NO: 23	AGC10	Forward primer
SEQ ID NO: 24	AGC10	Reverse primer

SEQ ID NO: 25	ACT1	Forward primer
SEQ ID NO: 26	ACT1	Reverse primer
SEQ ID NO: 27	CCT2	Forward primer
SEQ ID NO: 28	CCT2	Reverse primer

[0033] The polynucleotides of the present invention may be prepared by two general methods: (1) they may be synthesized from appropriate nucleotide triphosphates, or (2) they may be isolated from biological sources. Both methods utilize protocols well known in the art. The availability of nucleotide sequence information enables preparation of an isolated nucleic acid molecule of the invention by oligonucleotide synthesis. Synthetic oligonucleotides may be prepared by the phosphoramidite method employed in the Applied Biosystems 38A DNA Synthesizer or similar devices. The resultant construct may be purified according to methods known in the art, such as high performance liquid chromatography (HPLC). Complementary segments thus produced may be annealed such that each segment possesses appropriate cohesive termini for attachment of an adjacent segment. Adjacent segments may be ligated by annealing cohesive termini in the presence of DNA ligase to construct an entire long double-stranded molecule. A synthetic DNA molecule so constructed may then be cloned and amplified in an appropriate vector.

Total Genetic Diversity

[0034] A total of 295 *C. sativa* samples were analyzed and these samples included representatives from 33 countries or regions around the world. The greatest number of representative samples (188) came from the United States (Table 1). Virtually all of the samples in this study came either from drug confiscations or from known drug varieties of marijuana. Additionally, there were a small number of samples (< 10) that were from known hemp or fiber varieties of *Cannabis*. DNA extracted from four dried samples that came from drug confiscations conducted in 1992 were included in the analyses. Although the DNA was fairly degraded, complete genotypic profiles were obtained for each of these four samples.

[0035] 268 unique genotypes were found from the 295 *C. sativa* samples. For the samples that had at least one matching genotype from a different sample, it was noted that matches corresponded to samples with close geographic locations. All loci amplified robustly using 10 to 15ng DNA and exhibited Mendelian inheritance, with a maximum of two alleles per locus. A total of 63 alleles were detected in this data set, with the number of alleles per locus ranging from two at the AGC6 locus to 16 alleles at the AAAG1 locus (Table 2, Figure 2). The overall observed heterozygosity (averaged across loci) was 0.41±0.01 (mean±S.E.) while the expected heterozygosity was calculated to be 0.58±0.05, when averaged across all eight loci. The average heterozygosity per locus ranged from 0.21 to 0.79.

Allele Frequencies Per Locus

[0036] Figure 2 shows the allele frequencies for each locus in this data set. All observed alleles within each locus, with the exception of two loci, varied by the addition or deletion of single repeat motifs, which is consistent with the assumption that STR loci mutate by insertions and deletions of repeat units. Exceptions of this assumption were observed at the AAAG1 and AGC6 loci. The AAAG1 locus was isolated from a sequence that appeared to contain a 4 bp repeat motif however; samples subjected to the fragment analyses appeared to vary by 2 bp instead of four. The AGC6 locus only had two observable allele sizes, spanning 21bp, which would suggest a mutational event of seven repeat motif units.

[0037] The most diverse marker in this study was the AAAG1 locus, containing 16 alleles and spanning a 32 bp region of the genome, and all expected alleles were observed within this size range (Fig. 2). The second most diverse marker, AGC10 proved to be a noteworthy locus because of its large size range. At this locus we observed 15 alleles and an allelic size range from 273 bp to 336 (Table 2). All but seven of the 22 expected alleles were observed within this 63 bp size range.

Geographic Patterns

[0038] A neighbor-joining tree based on the proportion of shared alleles between samples was constructed. An assignment test was conducted to explore the potential utility of these markers for making geographic assignments based on a particular

genotype. The results suggest a possible utility of these markers in detecting geographic differences on large, regional scales such as continents. The results of the neighbor-joining tree (Fig. 3) depict large-scale geographic clustering based on similar genotypes. All states within North America clustered together. Additionally, samples from Europe and Asia clustered together, while samples from South America and Africa clustered together.

[0039] The results of the assignment test (Fig. 4) indicate that in general, genotypes can be correctly assigned to the right continent at least 50% of the time. Genotypes from the African population (13 samples) were correctly assigned to Africa in all instances; whereas genotypes from the Asian population (46 samples) were only correctly assigned to Asia 61% of the time (Table 1, Fig. 4). The North American population had the largest sample size (196 samples) and their genotypes were correctly assigned 72% of the time. This North American population, with its relatively large sample size, suggests that correct assignments to populations may increase with increasing sample size.

Genetic Diversity Among Individual Samples

[0040] We conducted an analysis of molecular variance (AMOVA) to determine the distribution of the genetic variation. Our findings revealed that the greatest proportion of genetic variation (~ 90%) was among individual samples, within counties and states (Table 3). While the AMOVA did indicate that there were significant differences ($P < 0.0001$) within countries and continents, this variation only accounted for approximately 8% of the total variance. This analysis also shows that the variation among the continents was not statistically significant at 2% (Table 3). The results of the AMOVA (Table 3) suggest that these markers are able to detect genetic differences between individual samples. Additionally, the number of unique genotypes observed, 268 out of 295 samples, also indicates that this system is capable of detecting a sizeable portion of the variation in the samples analyzed.

EXPERIMENTAL DETAILS

DNA Extraction and Sample Preparation

[0041] *Cannabis sativa* DNA was extracted from dried leaf and flower material, in crime laboratories independent of our laboratory, by criminalistics professionals licensed to legally handle these plant samples. Virtually all of the samples came from drug confiscations or from known drug varieties of marijuana. Four different crime laboratories provided DNA samples for this study and there were two main extraction protocols that these agencies used. From these laboratories, we obtained a total of 295 samples with a wide geographic distribution, including representative samples from five different continents (see Table 1). For samples within the United States, the sample location generally refers to the location of the drug confiscation and cultivation. However, the international sample locations do not necessarily correspond to the location of cultivation. Rather these locations correspond to region where the seeds were obtained.

[0042] The majority of samples (240 samples) were extracted by the Appalachian H.I.D.T.A. Marijuana Signature Laboratory, Frankfort, KY, using a modified CTAB (cetyltrimethylammonium bromide) protocol described by Weising *et al.* [15]. The remaining 55 samples were extracted in three independent laboratories, all using QIAGEN®'s DNeasy® plant mini kit (QIAGEN, Inc., Valencia, CA, USA), following manufacturers recommendations for dried plant material. DNA samples were received in 100-150 µl of TE buffer [10 mM tris-HCl at pH 8.0, 1 mM EDTA (ethylenediaminetetraacetic acid)] and stored at –20 °C. The approximate yield of each sample was assessed on a 0.7% agarose gel, where samples were compared to a Lambda Hind III DNA mass ladder of known concentrations (Invitrogen, Carlsbad, CA, USA). All DNA samples were then diluted to approximately 10 to 15 ng/ul for the subsequent analyses.

Development of STR Markers

[0043] The STR (microsatellite) markers were developed using a modified magnetic bead protocol that was first described by Li et al. [16] and modified by Pearson [17]. Genomic DNA was digested from three different marijuana plants using an *MboI* restriction enzyme (Invitrogen; Carlsbad, CA). Sau 3a I Linkers A and B (SAULA: 5'

GCG GTA CCC GGG AAG CTT GG 3' and SAULB: 5' GAT CCC AAG CTT CCC GGG TAC CGC 3') were ligated onto the digested genomic DNA and SAULA was used as a primer for subsequent polymerase chain reactions (PCR) [16]. The digested genomic DNA was amplified in multiple PCR reactions and concentrated to gain enough DNA for the following bead hybridization process.

[0044] Seven arbitrary repeat motifs were chosen as probes for the bead hybridization reactions based on a review by Cardle et al. [18] where they suggested that plants contain more AT-rich repeats than GC-rich repeats. The short tandem repeat (STR) probes were ordered from Integrated DNA Technologies (Coralville, IA, USA) with a biotin label on the 5' end of the probes [$(AGC)_8$, $(AAAG)_5$, $(CCT)_8$, $(AATT)_5$, $(ATT)_8$, $(GATA)_5$, $(ATGC)_5$]. These repeat probes were then added to a bead hybridization reaction to select for fragments of DNA that contain the repeat motif of the probe. The goal of this bead hybridization process was to allow the fragments containing repeats to anneal to the biotin-labeled probes. After the hybridization, the selected fragments were isolated from the rest of the genomic DNA using streptavidin coated magnetic beads, which bind to the biotin labeled probes. These fragments were then eluted and re-amplified using the SAULA primer in additional PCR reactions. The bead hybridization and PCR re-amplification processes were then repeated two additional times to enrich for genomic DNA containing the selected repeats.

[0045] Once the bead hybridization and selection process was completed, the repeat enriched DNA was then ligated into a pGEM-T vector from ProMega (Madison, WI, USA) in order to begin the sequencing phase of this protocol. The vectors were cloned into electrocompetent *E. coli* cells that were then plated onto selective media containing [0.1 mg/mL ampicillin, 0.05 mg/mL X-Gal, and 1mM IPTG] and positive clones were sequenced on an ABI PRISM® 377 DNA Sequencer (Applied Biosystems; Foster City, CA, USA). The sequencing reactions were standard 20 µl reactions using the ABI PRISM® BigDye™ Terminators sequencing kits (Applied Biosystems; Foster City, CA, USA) and 3.2 pmol of PCR product for template. Sequences containing repeat motifs and sufficient flanking sequence were used to design primers with PrimerSelect software (DNASTAR Inc.; Madison, WI, USA).

[0046] Thirty-three primer pairs were screened on 3% agarose gels against 24 samples from different locations to identify polymorphic markers. Of the 33 markers that were initially screened, fifteen were determined to be polymorphic and we obtained these 15 markers with fluorescent dye labels. The fluorescent markers were tested on an ABI PRISM® 377 DNA Sequencer (Applied Biosystems; Foster City, CA, USA) and seven of the 15 markers were eliminated due to problems with scoring or very low levels of polymorphism. The remaining eight markers (see Table 2) were tested in three multiplex reactions with two to four markers per mix and gels were run using GeneScan 2.1.1 (Applied Biosystems; Foster City, CA, USA) collection software on an ABI PRISM® 377 DNA Sequencer (Applied Biosystems; Foster City, CA, USA). Once multiplex reactions were optimized, 295 samples from individual plants were screened across all eight markers.

PCR Amplification and Fragment Analysis

[0047] The eight STR markers were optimized to amplify DNA in three 10 µl multiplex reactions (see Table 2). The multiplex mixes each contained approximately 10-15 ng of template from C. sativa in a 10 µl PCR including the following (final concentrations): 1X PCR buffer (Invitrogen; Carlsbad, CA, USA), 3 mM $MgCl_2$ (Invitrogen; Carlsbad, CA, USA), 200 µM dNTPs, 0.2 µM fluorescent forward primers, 0.2 µM unlabeled forward primers, 0.4 µM unlabeled reverse primers, and 1 unit Platinum DNA Taq Polymerase (Invitrogen; Carlsbad, CA, USA). Amplification reactions were then carried out in 96-well microplates in a DNA engine thermocycler (MJ Research, Inc.; Waltham, MA, USA) and the reaction contained a total of 35 cycles. The thermocycling conditions were as follows: an initial incubation of 95°C for 5 min, next a cycle of denaturing at 95°C for 30 sec, annealing at (59°C, 60°C, or 62°C) for 30 sec, and extending at 72°C for 30 sec, repeated for a total of 35 cycles, with a final extension of 72°C for 2 min, and ending with a holding temperature of 15°C.

[0048] The PCR products were then diluted 1:10 with E-pure® purified water in preparation for fragment analysis on the ABI PRISM® 377 DNA Sequencer (Applied Biosystems; Foster City, CA, USA). A size standard ladder mix was prepared with 0.75 µl deionized formamide, 0.25 µl of ROX labeled MapMarkers™1000 (BioVentures, Inc.;

Murfreesboro, TN, USA), and 0.1 µl of blue dextran loading dye (supplied with the ROX size ladder). Approximately 1 µl of the size standard ladder mix was added to 1 µl of the diluted amplification products and denatured at 95°C for 2 minutes. From this mixture, roughly 1.6 µl was loaded on a porous membrane comb (The Gel Company; San Francisco, CA, USA) and then electrophoresed in a 5% polyacrylamide gel on the ABI PRISM® 377 DNA Sequencer (Applied Biosystems; Foster City, CA, USA) for 3.5 hours.

Scoring of STR Loci and Data Analysis

[0049] Electrophoresis data was collected automatically with GeneScan™ 2.1.1 software (PE Applied Biosystems; Foster City, CA, USA); following collection, this software was also used to determine the allele sizes by implementing the local Southern method.

[0050] After initial scoring was completed, Genotyper™ software (Applied Biosystems; Foster City, CA, USA) was used to confirm the allele scores. Banding patterns of homozygous and heterozygous genotypes were consistent with that of a single peak for homozygotes and double peaks for heterozygotes. Once all of the data scoring was complete, random samples were re-amplified and independently re-run to assess reproducibility and confirm the scoring and banding patterns.

[0051] Statistical analyses of the data were performed using a multitude of different analysis packages. An Excel add-in called The Excel Microsatellite Toolkit V3.1 [19] was used to calculate the number of matching genotypes, number of alleles, allele frequencies, and observed and expected heterozygosity. A distance matrix was generated in MICROSAT [20] based on the proportion of shared alleles, which was then input into PHYLIP [21] to construct a phylogenetic tree using a neighbor-joining algorithm. Genetic differentiation among continents was calculated in Arlequin V2.0 [22] using an Analysis of Molecular Variance (AMOVA). Finally an assignment test was performed in GenAlEx V5 [23].

EXAMPLES

The following examples illustrate locus sequences for all fifteen polymorphic loci isolated from *Cannabis sativa*. **Forward** and **Reverse** primers are **underlined**. **Variable regions** are in **lower case**. *Most probes have an additional G added to the 5' end of the oligo to increase adenylation. All sequences are 5' → 3'

EXAMPLE 1

This example illustrates the amplicons produced during the amplification of STR locus **AAAG 1** with multiplex cocktails comprising primer pairs SEQ ID NO: 1 and SEQ ID NO: 2.

Sequence for **AAAG 1** locus:

GCGGTACCCGGGAAGCTTGGGATCTAAACTGAGAGGTGGGTTTTG**GTCAGAA AGCGAAGACCTTTAGA**CCCAATATGAAGGAG**aagaagaagaagaagaagaagaagaaa gaaagaaagaaagaaagaaag**AAAACACAGCTAGCAAAAGAA**GTAAAGACAGGCAG CCATC**ATTAATGGCAGAGAGATAGAGTGAGAAAGAGATAGAAAGGAGGAG AGAGAGAGAGATAGAGAGTACAAGAAAGAAAGAGCAAAGCCAAGCTTCCCG GGTACCGC

AAAG1F: GTCAGAAAGC GAAGACCTTT AGA [23bp]
AAAG1R: GTAAAGACAG GCAGCCATC [19bp]

AAAG1F (rev. comp.): TCTAAAGGTC TTCGCTTTCT GAC [23bp]
AAAG1R (rev. comp.): GATGGCTGCC TGTCTTTAC [19bp]

AAAG1 array: AAGAAGAAGA AGAAGAAGAA GAAGAAAGAA AGAAAGAAAG AAAGAAAG [48bp]
AAAG1 motif: (AAG)8 + (AAAG)6

AAAG1 amplicon: [275bp]

GCGGTACCCG GGAAGCTTGG GATCTAAACT GAGAGGTGGG
TTTTGGTCAG AAAGCGAAGA CCTTTAGACC CAATATGAAG
GAGAAGAAGA AGAAGAAGAA GAAGAAGAAA GAAAGAAAGA
AAGAAAGAAA GAAAACACAG CTAGCAAAAG AAGTAAAGAC
AGGCAGCCAT CATTAATGGC AGAGAGATAG AGTGAGAAAG
AGATAGAAAG GAGGAGAGAG AGAGAGATAG AGAGTACAAG
AAAGAAAGAG CAAAGCCAAG CTTCCCGGGT ACCGC

AAAG1 (reverse compliment): [275bp]

GCGGTACCCG GGAAGCTTGG CTTTGCTCTT TCTTTCTTGT ACTCTCTATC
TCTCTCTCTC TCCTCCTTTC TATCTCTTTC TCACTCTATC TCTCTGCCAT
TAATGATGGC TGCCTGTCTT TACTTCTTTT GCTAGCTGTG TTTTCTTTCT
TTCTTTCTTT CTTTCTTTCT TCTTCTTCTT CTTCTTCTTC TTCTCCTTCA
TATTGGGTCT AAAGGTCTTC GCTTTCTGAC CAAAACCCAC CTCTCAGTTT
AGATCCCAAG CTTCCCGGGT ACCGC

EXAMPLE 2

This example illustrates the amplicons produced during the amplification of STR locus **AAAG 5** with multiplex cocktails comprising primer pairs SEQ ID NO: 3 and SEQ ID NO: 4.

Sequence for **AAAG 5** locus:

GCGGTACCCGGGAAGCTTGGCATCAACTTGTCAAGCATTTAATATAAGATTG
GAATATATGTAACATC**TCAATTAATGCTTATAGCCCATATGTTTTCTACTA**
CTTCTTCTTTTTCAGTTGGTGTTATATAGCTTGATGATTACTTTCACGGTGT**aaa**
caaaagaagaagaaagaaagaaagaaagaaagaagACATGGGTTGAGCTGCTTCTGTATATG
TTGTTCCATGGA**AGAACAAGAAGAAACAAAGTATTCCTGAAGTTG**TGATAT
TTGTACCTTCATTGAAAATACCATTACAATCTGATCCCAAGCTTCCCGGGTAC
CGC

AAAG5F: TCAATTAATG CTTATAGCCC ATATGTTTTC TACTAC [36bp]
AAAG5R: AGAACAAGAA GAAACAAAGT ATTCCTGAAG TTG [33bp]

AAAG5F (rev. comp.): GTAGTAGAAA ACATATGGGC TATAAGCATT AATTGA [36bp]

AAAG5R (rev. comp.): CAACTTCAGG AATACTTTGT TTCTTCTTGT TCT [33bp]

AAAG5 array: AAACAAAAGA AGAAGAAAGA AAGAAAGAAA GAAAGAAG [48bp]

AAAG5 motif: (AAAC)1 + (AAAAG)1 + (AAG)2 + (AAAG)5 + (AAG)1

AAAG5 amplicon: [327bp]

GCGGTACCCG GGAAGCTTGG CATCAACTTG TCAAGCATTT AATATAAGAT TGGAATATAT GTAACATCTC AATTAATGCT TATAGCCCAT ATGTTTTCTA CTACTTCTTC TTTTTCAGTT GGTGTTATAT AGCTTGATGA TTACTTTCAC GGTGTAAACA AAAGAAGAAG AAAGAAAGAA AGAAAGAAAG AAGACATGGG TTGAGCTGCT TCTGTATATG TTGTTCCATG GAAGAACAAG AAGAAACAAA GTATTCCTGA AGTTGTGATA TTTGTACCTT CATTGAAAAT ACCATTACAA TCTGATCCCA AGCTTCCCGG GTACCGC

AAAG5 reverse compliment: [327bp]

GCGGTACCCG GGAAGCTTGG GATCAGATTG TAATGGTATT TTCAATGAAG GTACAAATAT CACAACTTCA GGAATACTTT GTTTCTTCTT GTTCTTCCAT GGAACAACAT ATACAGAAGC AGCTCAACCC ATGTCTTCTT TCTTTCTTTC TTTCTTTCTT CTTCTTTTGT TTACACCGTG AAAGTAATCA TCAAGCTATA TAACACCAAC TGAAAAAGAA GAAGTAGTAG AAAACATATG GGCTATAAGC ATTAATTGAG ATGTTACATA TATTCCAATC TTATATTAAA TGCTTGACAA GTTGATGCCA AGCTTCCCGG GTACCGC

EXAMPLE 3

This example illustrates the amplicons produced during the amplification of STR locus **AAAG 6** with multiplex cocktails comprising primer pairs SEQ ID NO: 5 and SEQ ID NO: 6.

Sequence for **AAAG 6** locus:

GCGGTACCCGGGAAGCTTGGCTTAGATTAAGAATATTTGTAGTTTCGTACTTG
TATTCCTTGCCTTTTTCAAGATTTCTT
GCTTGTTTAGGGTATCTGCCATTTTTCTTTCTCCTTTCAGAGCTTCTTCTAATC
CAAGATTCCCAAGATGAGCAATTGTC
TTTTCACCCCACAGACTGAAATTGTT**TTTGCCATTGATTTCCTCCTCCTCAT**
ACTTCTCCAAAGACATTATTGAACAAATAAG**aaagaaagaaagaaagaaagaaagaaaga**
aagaaagAAAAACTTATGGCCAGTAAGCGTTTCCCTTGTTGGTTACCTTTCTTCA
GTCTTTGAGGAATTCATTCGAACACTCTGTCAACCTCAACTGGTTTCTTCAAA
CTCTAATCTGAAACCTGGCTCTTGATACCAGTTTGTGAGGATTGGTCTCCTCT
TCTCCAATCTC**AGATCCCAAGCTTCCCGGGTACC**GC

AAAG6F: TTTGCCATTG ATTTCCTCCT CCTCATAC [28bp]
AAAG6R: AGATCCCAAG CTTCCCGGGT ACC [23bp]
AAAG6F (rev. comp.): GTATGAGGAG GAGGAAATCA ATGGCAAA [28bp]
AAAG6R (rev. comp.): GGTACCCGGG AAGCTTGGGA TCT [23bp]
AAAG6 array: AAAGAAAGAA AGAAAGAAAG AAAGAAAGAA AGAAAG [36bp]
AAAG6 motif: (AAAG)9

AAAG6 locus: [469bp]
GCGGTACCCG GGAAGCTTGG CTTAGATTAA GAATATTTGT AGTTTCGTAC
TTGTATTCCT TGCCTTTTTC AAGATTTCTT GCTTGTTTAG GGTATCTGCC
ATTTTTCTTT CTCCTTTCAG AGCTTCTTCT AATCCAAGAT TCCCAAGATG
AGCAATTGTC TTTTCACCCC ACAGACTGAA ATTGTTTTTG CCATTGATTT
CCTCCTCCTC ATACTTCTCC AAAGACATTA TTGAACAAAT
AAGAAAGAAA GAAAGAAAGA AAGAAAGAAA GAAAGAAAGA
AAAACTTATG GCCAGTAAGC GTTTCCCTTG TTGGTTACCT TTCTTCAGTC

TTTGAGGAAT TCATTCGAAC ACTCTGTCAA CCTCAACTGG TTTCTTCAAA
CTCTAATCTG AAACCTGGCT CTTGATACCA GTTTGTGAGG ATTGGTCTCC
TCTTCTCCAA TCTCAGATCC CAAGCTTCCC GGGTACCGC

AAAG6 reverse compliment: [469bp]

GCGGTACCCG GGAAGCTTGG GATCTGAGAT TGGAGAAGAG
GAGACCAATC CTCACAAACT GGTATCAAGA GCCAGGTTTC
AGATTAGAGT TTGAAGAAAC CAGTTGAGGT TGACAGAGTG
TTCGAATGAA TTCCTCAAAG ACTGAAGAAA GGTAACCAAC
AAGGGAAACG CTTACTGGCC ATAAGTTTTT CTTTCTTTCT TTCTTTCTTT
CTTTCTTTCT TTCTTTCTTA TTTGTTCAAT AATGTCTTTG GAGAAGTATG
AGGAGGAGGA AATCAATGGC AAAAACAATT TCAGTCTGTG
GGGTGAAAAG ACAATTGCTC ATCTTGGGAA TCTTGGATTA
GAAGAAGCTC TGAAAGGAGA AAGAAAAATG GCAGATACCC
TAAACAAGCA AGAAATCTTG AAAAAGGCAA GGAATACAAG
TACGAAACTA CAAATATTCT TAATCTAAGC CAAGCTTCCC GGGTACCGC

EXAMPLE 4

This example illustrates the amplicons produced during the amplification of STR locus **AAAG 7** with multiplex cocktails comprising primer pairs SEQ ID NO: 7 and SEQ ID NO: 8.

Sequence for **AAAG 7** locus:

GCGGTACCCGGGAAGCTTGGATCAGAAAGACAAGACAAGATAGGGACTA**CT**
ACAAAGATTCCCACACTCAATAATGCAAATACAATTATTAGTACTAATAAT
GAAAACAACATCAAATTAAAGAAAAACCATAGAAG**aaaacaaaaagaaaagaaagaaa**
gaaagaaagATAGATAGATACCTGGTAGTGGGTTGGTTGGTTGGTGGTGATGAGT
ACTGAAATGGAAGACAATGAAAGGAGAAGGGGTTTACAGTGTTAACACTAT
AGTAAGGATTTGGTTTTCGGCTTTCGTTCTTTTAAGGAAGATGGGTGTTTG
AGAATGGATTGAGTAGTACAAGTCCAAATTCACAAGCAATTGCAGAGGCAGA
CGATGACTTCTTCAAATTCATAAGCAAGTGCCGAGGCAACCGATCCCAAGCT
TCCCGGGTACCGC

AAAG7F: CTACAAAGAT TCCCACACTC AATAATGCAA ATACAA [36bp]

AAAG7R: AGTAAGGATT TGGTTTTCGG CTTTCGTTCT T [31bp]

AAAG7F (rev. comp.): TTGTATTTGC ATTATTGAGT GTGGGAATCT TTGTAG [36bp]

AAAG7R (rev. comp.): AAGAACGAAA GCCGAAAACC AAATCCTTAC T[31bp]

AAAG7 array: AAAACAAAAA GAAAAGAAAG AAAGAAAGAA AG [32bp]

AAAG7 motif: (AAAAAG)1 + (AAAAG)1 + (AAAG)4

AAAG7 locus: [434bp]

GCGGTACCCG GGAAGCTTGG ATCAGAAAGA CAAGACAAGA
TAGGGACTAC TACAAAGATT CCCACACTCA ATAATGCAAA
TACAATTATT AGTACTAATA ATGAAAACAA CATCAAATTA
AAGAAAAACC ATAGAAGAAA ACAAAAAGAA AAGAAAGAAA
GAAAGAAAGA TAGATAGATA CCTGGTAGTG GGTTGGTTGG
TTGGTGGTGA TGAGTACTGA AATGGAAGAC AATGAAAGGA
GAAGGGGTTT ACAGTGTTAA CACTATAGTA AGGATTTGGT TTTCGGCTTT
CGTTCTTTTA AGGAAGATGG GTGTTTGAGA ATGGATTGAG
TAGTACAAGT CCAAATTCAC AAGCAATTGC AGAGGCAGAC
GATGACTTCT TCAAATTCAT AAGCAAGTGC
CGAGGCAACC GATCCCAAGC TTCCCGGGTA CCGC

AAAG7 reverse compliment: [434bp]

GCGGTACCCG GGAAGCTTGG GATCGGTTGC CTCGGCACTT
GCTTATGAAT TTGAAGAAGT CATCGTCTGC CTCTGCAATT GCTTGTGAAT
TTGGACTTGT ACTACTCAAT CCATTCTCAA ACACCCATCT TCCTTAAAAG
AACGAAAGCC GAAAACCAAA TCCTTACTAT AGTGTTAACA
CTGTAAACCC CTTCTCCTTT CATTGTCTTC CATTTCAGTA CTCATCACCA
CCAACCAACC AACCCACTAC CAGGTATCTA TCTATCTTTC TTTCTTTCTT
TCTTTTCTTT TTGTTTTCTT CTATGGTTTT TCTTTAATTT GATGTTGTTT
TCATTATTAG TACTAATAAT TGTATTTGCA TTATTGAGTG TGGGAATCTT
TGTAGTAGTC CCTATCTTGT CTTGTCTTTC TGATCCAAGC TTCCCGGGTA
CCGC

EXAMPLE 5

This example illustrates the amplicons produced during the amplification of STR locus **AAAG 10** with multiplex cocktails comprising primer pairs SEQ ID NO: 9 and SEQ ID NO: 10.

Sequence for **AAAG 10** locus:

GCGGTACCCGGGAAGCTTGGATAA**CAAAAATTCATACATAAGGCACGAAG AGATAGACA**TAG**aaagaaagaaagaaagaaag**GAAAAAAAAAAATACTAAAACGAC ATACACGGTCTTAGAGGACGAAGCAACTGCGCCGCCGCCGGTGACTGGGTTCC CT

TGGTCGAGAGGGAAAAAGAGGTTTTTGGTCTCTCTGACTCTGTTGTGCAGTGA GATGAGGAGTGGAGAGTCGGATAGCATCATTTTTACACTAACTGAGAAGAAC AACTTTTGATTTGGTTTGGTTTAAGGAAGAAAAAATCCCACATCGACTTGTTA TAGCTTTTTTAATATGTTTATATTGATTAC**TTTATACAGTCCTATCGCCGGG TCCAA**GCTTCCCGGGTACCGC

AAAG10F: CAAAAATTCA TACATAAGGC ACGAAGAGAT AGACA [35bp]
AAAG10R: TTTATACAGT CCTATCGCCG GGTCCAA [27bp]

AAAG10F (rev. comp.): TGTCTATCTC TTCGTGCCTT ATGTATGAAT TTTTG [35bp]
AAAG10R (rev. comp.): TTGGACCCGG CGATAGGACT GTATAAA [27bp]

AAAG10 array: AAAGAAAGAA AGAAAGAAAG [20bp]
AAAG10 motif: (AAAG)5

AAAG10 locus: [391bp]
GCGGTACCCG GGAAGCTTGG ATAACAAAAA TTCATACATA
AGGCACGAAG AGATAGACAT AGAAAGAAAG AAAGAAAGAA
AGGAAAAAAA AAAATACTAA AACGACATAC ACGGTCTTAG
AGGACGAAGC AACTGCGCCG CCGCCGGTGA CTGGGTTCCT
TGGTCGAGAG GGAAAAAGAG GTTTTTGGTC TCTCTGACTC TGTTGTGCAG

TGAGATGAGG AGTGGAGAGT CGGATAGCAT CATTTTTACA
CTAACTGAGA AGAACAACTT TTGATTTGGT TTGGTTTAAG
GAAGAAAAAA TCCCACATCG ACTTGTTATA GCTTTTTTAA TATGTTTATA
TTGATTACTT TATACAGTCC TATCGCCGGG TCCAAGCTTC CCGGGTACCG
C

AAAG10 reverse compliment: [391bp]
GCGGTACCCG GGAAGCTTGG ACCCGGCGAT AGGACTGTAT
AAAGTAATCA ATATAAACAT ATTAAAAAAG CTATAACAAG
TCGATGTGGG ATTTTTTCTT CCTTAAACCA AACCAAATCA AAAGTTGTTC
TTCTCAGTTA GTGTAAAAAT GATGCTATCC GACTCTCCAC TCCTCATCTC
ACTGCACAAC AGAGTCAGAG AGACCAAAAA CCTCTTTTTC
CCTCTCGACC AAGGAACCCA GTCACCGGCG GCGGCGCAGT
TGCTTCGTCC TCTAAGACCG TGTATGTCGT TTTAGTATTT TTTTTTTTCC
TTTCTTTCTT TCTTTCTTTC TATGTCTATC TCTTCGTGCC TTATGTATGA
ATTTTTGTTA TCCAAGCTTC CCGGGTACCG C

EXAMPLE 6

This example illustrates the amplicons produced during the amplification of STR locus **AAAG 11** with multiplex cocktails comprising primer pairs SEQ ID NO: 11 and SEQ ID NO: 12.

Sequence for **AAAG 11** locus:
TTGCGGTACCCGGGAAGCTTGGATCTTAAAAGTTCAGGGGGCAAAAATCATA
ATTAGCCTATTGTTAATAATAGACCCTCCTAAAAATCGTTTTGCAAAATAACA
TTC**TTTTCATAATTGTTTGCAAAATAATCTTTCTCTAGAA**TCCAAATAGTAT
TGAGAATTTTTAACAAAGTATTTGGAATTCTTAACAAAATGTTAGATTGTGAA
GGTGCTAGAAAGGTCATTTTTTGTTAAAAATTATCATCTATCAATTACTCATG
ATAGATTGTTGGAATAGAATCACAAGTTTTTGTTACACTATTATGTGGAGTGA
TTGGTGAAAATACACTTATTATGCAAATTGTACATAAAAAGAAGG**aaagaaagaa**
agaaagTCTATTTCACCAAACAAAAGAAACACCTTTATTATGTGAAAGTGATTG
ATGCATAAAGACTAATAATGCAGGATTTGAAGAGCCTTTGAGAGCAT**GTTGT**

GGTCATGGTGGGAAGTATAATTTTAATAAGAaCATTGGATGTGGGGGCAAG AAAATGGTCCATGGGAAAGAGATTTTGGTGGGAAAGGCTTGTAAAGATCCAA GCTTCCCGGGTACCGC

AAAG11F: TTTTCATAAT TGTTTGCAAA ATAATCTTTC TCTAGAA [37bp]
AAAG11R: GTTGTGGTCA TGGTGGGAAG TATAATTTTA ATA [33bp]

AAAG11F (rev. comp.): TTCTAGAGAA AGATTATTTT GCAAACAATT ATGAAAA [37bp]
AAAG11R (rev. comp.): TATTAAAATT ATACTTCCCA CCATGACCAC AAC [33bp]

AAAG11 array: AAAGAAAGAA AGAAAG [16bp]
AAAG11 motif: (AAAG)4

AAAG11 locus: [596bp]
TTGCGGTACC CGGGAAGCTT GGATCTTAAA AGTTCAGGGG GCAAAAATCA TAATTAGCCT ATTGTTAATA ATAGACCCTC CTAAAAATCG TTTTGCAAAA TAACATTCTT TTCATAATTG TTTGCAAAAT AATCTTTCTC TAGAATCCAA ATAGTATTGA GAATTTTTAA CAAAGTATTT GGAATTCTTA ACAAAATGTT AGATTGTGAA GGTGCTAGAA AGGTCATTTT TTGTTAAAAA TTATCATCTA TCAATTACTC ATGATAGATT GTTGGAATAG AATCACAAGT TTTTGTTACA CTATTATGTG GAGTGATTGG TGAAAATACA CTTATTATGC AAATTGTACA TAAAAAGAAG GAAAGAAAGA AAGAAAGTCT ATTTCACCAA ACAAAAGAAA CACCTTTATT ATGTGAAAGT GATTGATGCA TAAAGACTAA TAATGCAGGA TTTGAAGAGC CTTTGAGAGC ATGTTGTGGT CATGGTGGGA AGTATAATTT TAATAAGAAC ATTGGATGTG GGGGCAAGAA AATGGTCCAT GGGAAAGAGA TTTTGGTGGG AAAGGCTTGT AAAGATCCAA GCTTCCCGGG TACCGC

AAAG11 reverse compliment: [596bp]

GCGGTACCCG GGAAGCTTGG ATCTTTACAA GCCTTTCCCA CCAAAATCTC
TTTCCCATGG ACCATTTTCT TGCCCCCACA TCCAATGTTC TTATTAAAAT
TATACTTCCC ACCATGACCA CAACATGCTC TCAAAGGCTC TTCAAATCCT
GCATTATTAG TCTTTATGCA TCAATCACTT TCACATAATA AAGGTGTTTC
TTTTGTTTGG TGAAATAGAC TTTCTTTCTT TCTTTCCTTC TTTTTATGTA
CAATTTGCAT AATAAGTGTA TTTTCACCAA TCACTCCACA TAATAGTGTA
ACAAAAACTT GTGATTCTAT TCCAACAATC TATCATGAGT AATTGATAGA
TGATAATTTT TAACAAAAAA TGACCTTTCT AGCACCTTCA CAATCTAACA
TTTTGTTAAG AATTCCAAAT ACTTTGTTAA AAATTCTCAA TACTATTTGG
ATTCTAGAGA AAGATTATTT TGCAAACAAT TATGAAAAGA ATGTTATTTT
GCAAAACGAT TTTTAGGAGG GTCTATTATT AACAATAGGC TAATTATGAT
TTTTGCCCCC TGAACTTTTA AGATCCAAGC TTCCCGGGTA CCGCAA

EXAMPLE 7

This example illustrates the amplicons produced during the amplification of STR ocus **AGC 1** with multiplex cocktails comprising primer pairs SEQ ID NO: 13 and SEQ ID NO: 14.

Sequence for **AGC 1** locus:

GGGCCCGACGTCGCATGCTCCCGGCCGCCATGGCCGCGGGATTTACCCGGGA
AGCTTGGATAAGACCATGGCAAGAAAAGATGAGCAACAGAATGTGGTAATT
CAATACAAACAGAACACAAGTCGAATGGATAATAATAATAAGAAGAAACAG
TTGCCAAGCTGTCAAAAGAAATCACAGAACAATTTAGAGTTACAACAACCAT
TCGTGCCTGGAAAATTAGTATCACAAGATAATGGAAAACAAGTTTTACAGAC
AAGAAAACAAAAGGGTAGCACTGGTAGTAGTGAAGTTATGG**CAAAGAGTGT**
ATCGAAACCTGTCCGTGATGGAACAAATTTTCAACAGA**agcagcagcagcagcagca**
gcagcagcagcCACAGTCTAACCAAGAAAAGTTGAATAAGAAAGGTTTGAAAAAA
G**GTACTAATACAGACGATGTGGTGGG**GGTAGAAAGAAATTTGGCTGAATC
CAATTTCGTTAAGGAATACAACAATCGAAGCCCGGATCCCAAGCTTCCCGGG
TACCGC

AGC1F: CAAAGAGTGT ATCGAAACCT GTC [23bp]

AGC1R: GTACTAATAC AGACGATGTG GTGGG [25bp]

AGC1F (rev. comp.): GACAGGTTTC GATACACTCT TTG [23bp]
AGC1R (rev. comp.): CCCACCACAT CGTCTGTATT AGTAC [25bp]

AGC1 array: AGCAGCAGCA GCAGCAGCAG CAGCAGCAGC [30bp]
AGC1 motif: (AGC)10

AGC1 locus: [529bp]
GGGCCCGACG TCGCATGCTC CCGGCCGCCA TGGCCGCGGG
ATTTACCCGG GAAGCTTGGA TAAGACCATG GCAAGAAAAG
ATGAGCAACA GAATGTGGTA ATTCAATACA AACAGAACAC
AAGTCGAATG GATAATAATA ATAAGAAGAA ACAGTTGCCA
AGCTGTCAAA AGAAATCACA GAACAATTTA GAGTTACAAC
AACCATTCGT GCCTGGAAAA TTAGTATCAC AAGATAATGG
AAAACAAGTT TTACAGACAA GAAAACAAAA GGGTAGCACT
GGTAGTAGTG AAGTTATGGC AAAGAGTGTA TCGAAACCTG
TCCGTGATGG AACAAATTTT CAACAGAAGC AGCAGCAGCA
GCAGCAGCAG CAGCAGCCAC AGTCTAACCA AGAAAAGTTG
AATAAGAAAG GTTTGAAAAA AGGTACTAAT ACAGACGATG
TGGTGGGGGT AGAAAGAAAT TTGGCTGAAT CCAATTTCGT
TAAGGAATAC AACAATCGAA GCCCGGATCC CAAGCTTCCC GGGTACCGC

AGC1 reverse compliment: [529bp]
GCGGTACCCG GGAAGCTTGG GATCCGGGCT TCGATTGTTG TATTCCTTAA
CGAAATTGGA TTCAGCCAAA TTTCTTTCTA CCCCCACCAC ATCGTCTGTA
TTAGTACCTT TTTTCAAACC TTTCTTATTC AACTTTTCTT GGTTAGACTG
TGGCTGCTGC TGCTGCTGCT GCTGCTGCTG CTTCTGTTGA AAATTTGTTC
CATCACGGAC AGGTTTCGAT ACACTCTTTG CCATAACTTC ACTACTACCA
GTGCTACCCT TTTGTTTTCT TGTCTGTAAA ACTTGTTTTC CATTATCTTG
TGATACTAAT TTTCCAGGCA CGAATGGTTG TTGTAACTCT AAATTGTTCT
GTGATTTCTT TTGACAGCTT GGCAACTGTT TCTTCTTATT ATTATTATCC

ATTCGACTTG TGTTCTGTTT GTATTGAATT ACCACATTCT GTTGCTCATC TTTTCTTGCC ATGGTCTTAT CCAAGCTTCC CGGGTAAATC CCGCGGCCAT GGCGGCCGGG AGCATGCGAC GTCGGGCCC

EXAMPLE 8

This example illustrates the amplicons produced during the amplification of STR locus **AGC 3** with multiplex cocktails comprising primer pairs SEQ ID NO: 15 and SEQ ID NO: 16.

Sequence for **AGC 3** locus:

GCGGTACCCGGGAAGCTTGGATCCTGGTAAAATAAAATTCCAACAGTTCACA AGTACCAAACACAACTCCCCCTGGAAAAGGGTCAAGATTTTGTCCAAACAAA CAGTTAAAAATCAAAATATTACTCCCCCTTTTTGTTTATCTAAGGGCCAAAGA TAACAAACATGAAAAT**ATAGTAATATGTCCAACAAAAGCAAAGAAAGAAA AA**AAAACTTAGTCTCTGTAAAGCTTGACCAAGGTGGACAACTGCTTTGACAT CTTTTGCTGAACTTCCTCCATGGCAGCAAGACGATTGTTCACCAGCTGAACCT CATTCTTGACGTCATGGATTTCTGCGGAAGCAGAATTCGAGCTTGCAA**Cagcag cagcagcaccagc**TTTAGGCCATTTTTGAAACACACCATCAAAGTATTTCGAGGGTT GGAATGTAGGTCCAATGATAGGGGGCT**CAAGTGTTTCATGTGATTGGGCCA C**ATTCTTTTGGGAAGATAAAACCTTATAGATTAGATTTGGAAATACAAGTTTA AAGGTTGGCTTTTTATCTCTTCGGAAAGAAACAATCTGGTTCAGAATGTGTGA GGCCAAATCAATTGAAGCTCCAGAGGTGATGCGGTATAAGAATGATGCCACA TCTTGAGACACTACGGTCTTGTTGGAGT

AGC3F: ATAGTAATAT GTCCAACAAA AGCAAAGAAA GAAAAA [36bp]

AGC3R: CAAGTGTTTC ATGTGATTGG GCCAC [25bp]

AGC3F (rev. comp.): TTTTTCTTTC TTTGCTTTTG TTGGACATAT TACTAT [36bp]

AGC3R (rev. comp.): GTGGCCCAAT CACATGAAAC ACTTG [25bp]

AGC3 array: AGCAGCAGCA GCACCAGC [18bp]

AGC3 motif: (AGC)6

AGC3 locus: [660bp]

GCGGTACCCG GGAAGCTTGG ATCCTGGTAA AATAAAATTC
CAACAGTTCA CAAGTACCAA ACACAACTCC CCCTGGAAAA
GGGTCAAGAT TTTGTCCAAA CAAACAGTTA AAAATCAAAA
TATTACTCCC CCTTTTTGTT TATCTAAGGG CCAAAGATAA CAAACATGAA
AATATAGTAA TATGTCCAAC AAAAGCAAAG AAAGAAAAAA
AAACTTAGTC TCTGTAAAGC TTGACCAAGG TGGACAACTG CTTTGACATC
TTTTGCTGAA CTTCCTCCAT GGCAGCAAGA CGATTGTTCA CCAGCTGAAC
CTCATTCTTG ACGTCATGGA TTTCTGCGGA AGCAGAATTC GAGCTTGCAA
CAGCAGCAGC AGCACCAGCT TTAGGCCATT TTTGAAACAC
ACCATCAAAG TATTTCGAGG GTTGGAATGT AGGTCCAATG
ATAGGGGGCT CAAGTGTTTC ATGTGATTGG GCCACATTCT TTTGGGAAGA
TAAAACCTTA TAGATTAGAT TTGGAAATAC AAGTTTAAAG GTTGGCTTTT
TATCTCTTCG GAAAGAAACA ATCTGGTTCA GAATGTGTGA
GGCCAAATCA ATTGAAGCTC CAGAGGTGAT GCGGTATAAG
AATGATGCCA CATCTTGAGA CACTACGGTC TTGTTGGAGT

AGC3 reverse compliment: [660bp]

ACTCCAACAA GACCGTAGTG TCTCAAGATG TGGCATCATT CTTATACCGC
ATCACCTCTG GAGCTTCAAT TGATTTGGCC TCACACATTC TGAACCAGAT
TGTTTCTTTC CGAAGAGATA AAAAGCCAAC CTTTAAACTT GTATTTCCAA
ATCTAATCTA TAAGGTTTTA TCTTCCCAAA AGAATGTGGC CCAATCACAT
GAAACACTTG AGCCCCCTAT CATTGGACCT ACATTCCAAC CCTCGAAATA
CTTTGATGGT GTGTTTCAAA AATGGCCTAA AGCTGGTGCT GCTGCTGCTG
TTGCAAGCTC GAATTCTGCT TCCGCAGAAA TCCATGACGT
CAAGAATGAG GTTCAGCTGG TGAACAATCG TCTTGCTGCC
ATGGAGGAAG TTCAGCAAAA GATGTCAAAG CAGTTGTCCA
CCTTGGTCAA GCTTTACAGA GACTAAGTTT TTTTTTCTTT CTTTGCTTTT
GTTGGACATA TTACTATATT TTCATGTTTG TTATCTTTGG CCCTTAGATA
AACAAAAAGG GGGAGTAATA TTTTGATTTT TAACTGTTTG TTTGGACAAA

ATCTTGACCC TTTTCCAGGG GGAGTTGTGT TTGGTACTTG TGAACTGTTG GAATTTTATT TTACCAGGAT CCAAGCTTCC CGGGTACCGC

EXAMPLE 9

This example illustrates the amplicons produced during the amplification of STR locus **AGC 6** with multiplex cocktails comprising primer pairs SEQ ID NO: 17 and SEQ ID NO: 18.

Sequence for **AGC 6** locus:

TACWTGAGCCCGACGTCGCATGCTCCCGGCCGCCATGGCCCGCGGGATTGCG
GTACCCGGGAAGCTTGGCAATATACAATCTSAGKTCACTCTCTGCTTTCCCAA
GCAGCCCTTGTTTGCAAGTATGCTCAAGACCAACGAAGTACCAGCACTGAGG
CTTGAATGCATGAGTAAAATGTAAAGAAGCCTTCTTTCCCTTTCCGCTTCCAC
TTTCCACCACCAAAAACTGTGCATGGAAGTATGCCTCTATTCCCTGGTTGTCA
GCAGACAAGAAACTGAAC**AGACGTGGCATATGCGCTGTTCCTTCA**CCTGC
AAGCGCACTGGCAGCAGCAGCAGCCGACATAGCTGAAGATTTTCCTGACTT**ag**
cagcagcagcagcagcTATTGCAGCAGCAGCAGTTGCTGTATTTAACGTATCAGCAA
ATGATTCAATGTAAATCCATGTTGCAAAT**GCATACCCATTAGTGAACGGCC**
ATCGGCTTTCCCCTGGACCAAGCAAACCAGAGCTTTCACCATCAAACTCAAA
AGTACATGCTGGTCCCTTTGACTCCTTTCCACTAACTGCCTTCTCCAAAGCAA
TCATTAAGCGAGCTGACCAAACAGTGCTAAGTGTTCTTGTGATGACTTGAAA
CCATCTATGCAAATCGATGACACTAAGTG

AGC6F: AGACGTGGCA TATGCGCTGT TCCTTCA [27bp]
AGC6R: GCATACCCAT TAGTGAACGG CCATCGGC [28bp]

AGC6F (rev. comp.): TGAAGGAACA GCGCATATGC CACGTCT [27bp]
AGC6R (rev. comp.): GCCGATGGCC GTTCACTAAT GGGTATGC [28bp]

AGC6 array: AGCAGCAGCA GCAGCAGC [18bp]
AGC6 motif: (AGC)6

AGC6 locus: [663bp]

TACWTGAGCC CGACGTCGCA TGCTCCCGGC CGCCATGGCC
CGCGGGATTG CGGTACCCGG GAAGCTTGGC AATATACAAT
CTSAGKTCAC TCTCTGCTTT CCCAAGCAGC CCTTGTTTGC AAGTATGCTC
AAGACCAACG AAGTACCAGC ACTGAGGCTT GAATGCATGA
GTAAAATGTA AAGAAGCCTT CTTTCCCTTT CCGCTTCCAC TTTCCACCAC
CAAAAACTGT GCATGGAAGT ATGCCTCTAT TCCCTGGTTG TCAGCAGACA
AGAAACTGAA CAGACGTGGC ATATGCGCTG TTCCTTCACC
TGCAAGCGCA CTGGCAGCAG CAGCAGCCGA CATAGCTGAA
GATTTTCCTG ACTTAGCAGC AGCAGCAGCA GCTATTGCAG
CAGCAGCAGT TGCTGTATTT AACGTATCAG CAAATGATTC AATGTAAATC
CATGTTGCAA ATGCATACCC ATTAGTGAAC GGCCATCGGC TTTCCCCTGG
ACCAAGCAAA CCAGAGCTTT CACCATCAAA CTCAAAAGTA
CATGCTGGTC CCTTTGACTC CTTTCCACTA ACTGCCTTCT CCAAAGCAAT
CATTAAGCGA GCTGACCAAA CAGTGCTAAG TGTTCTTGTG
ATGACTTGAA ACCATCTATG CAAATCGATG ACACTAAGTG AGC

AGC6 reverse compliment: [663bp]

GCTCACTTAG TGTCATCGAT TTGCATAGAT GGTTTCAAGT
CATCACAAGA ACACTTAGCA CTGTTTGGTC AGCTCGCTTA ATGATTGCTT
TGGAGAAGGC AGTTAGTGGA AAGGAGTCAA AGGGACCAGC
ATGTACTTTT GAGTTTGATG GTGAAAGCTC TGGTTTGCTT GGTCCAGGGG
AAAGCCGATG GCCGTTCACT AATGGGTATG CATTTGCAAC ATGGATTTAC
ATTGAATCAT TTGCTGATAC GTTAAATACA GCAACTGCTG
CTGCTGCAAT AGCTGCTGCT GCTGCTGCTA AGTCAGGAAA ATCTTCAGCT
ATGTCGGCTG CTGCTGCTGC CAGTGCGCTT GCAGGTGAAG
GAACAGCGCA TATGCCACGT CTGTTCAGTT TCTTGTCTGC TGACAACCAG
GGAATAGAGG CATACTTCCA TGCACAGTTT TTGGTGGTGG
AAAGTGGAAG CGGAAAGGGA AAGAAGGCTT CTTTACATTT
TACTCATGCA TTCAAGCCTC AGTGCTGGTA CTTCGTTGGT CTTGAGCATA
CTTGCAAACA AGGGCTGCTT GGGAAAGCAG AGAGTGAMCT

SAGATTGTAT ATTGCCAAGC TTCCCGGGTA CCGCAATCCC GCGGGCCATG GCGGCCGGGA GCATGCGACG TCGGGCTCAW GTA

EXAMPLE 10

This example illustrates the amplicons produced during the amplification of STR locus **AGC 8** with multiplex cocktails comprising primer pairs SEQ ID NO: 19 and SEQ ID NO: 20.

Sequence for **AGC 8** locus:

GCGGTACCCGGGAAGCTTGGATCCCAAGATCCCCTACCTCTTTCGTTCTGAGG
CACGCCAGAAGATTTAGAAGTATCAATAGCTCCAAATTCAGAAGAGACACCT
CTGTTAACGGCGTGTCTAAGGTTCCC**TTCCGACACCGGCGACGCACTC**GAG
CTCCATACGAACATATGAAGGTCCTTGTTCGGCAGACCATTATT**agcagcagcagca**
gcaggaggaggTGCTGTAACAGTTGTTGCGTCTTTCTTCTTAACAGCCGTATTACTT
GTCGACCCGGAAAACATCGGATTAGGAGGAGGGTAAGACGGGGCAAGACCG
CCATTGAAGAGCTCTCCACTCATGCTCCTCGCTCCTCTCTGC**TTCTTTCCCAT**
ATTTTTCATCATCTCTTCGTCGAAATTAGATGTCCTTGGCGTGACGCCTTTC
GATGACTGAAGTGAGTAGACATCAGCGCCGTGAGTTGGTCCACCACCGTAGC
TGTTGGTGTACCCGTGTTTGGGACTAGCGGCCTTACTGGCATTAAACATGGCG
TAAAAATCAGTCTGGTTGAAGCTCGATGCCCTCGGGGTCGGCTCTCGCGAGG
ATTGTACAGAGTAGATCCCAAGCTTCCCGGGTACCGC

AGC8F: TTCCGACACC GGCGACGCAC TC [22bp]
AGC8R: TTCTTTCCCA TATTTTTCAT CATCTCTTCG TCGAA [35bp]

AGC8F (rev. comp.): GAGTGCGTCG CCGGTGTCGG AA [22bp]
AGC8R (rev. comp.): TTCGACGAAG AGATGATGAA AAATATGGGA AAGAA [35bp]

AGC8 array: AGCAGCAGCA GCAGCAGGAG GAGG [28bp]
AGC8 motif: (AGC)5 + (AGG)3

AGC8 locus: [620bp]

GCGGTACCCG GGAAGCTTGG ATCCCAAGAT CCCCTACCTC TTTCGTTCTG
AGGCACGCCA GAAGATTTAG AAGTATCAAT AGCTCCAAAT
TCAGAAGAGA CACCTCTGTT AACGGCGTGT CTAAGGTTCC CTTCCGACAC
CGGCGACGCA CTCGAGCTCC ATACGAACAT ATGAAGGTCC
TTGTTCGGCA GACCATTATT AGCAGCAGCA GCAGCAGGAG
GAGGTGCTGT AACAGTTGTT GCGTCTTTCT TCTTAACAGC CGTATTACTT
GTCGACCCGG AAAACATCGG ATTAGGAGGA GGGTAAGACG
GGGCAAGACC GCCATTGAAG AGCTCTCCAC TCATGCTCCT CGCTCCTCTC
TGCTTCTTTC CCATATTTTT CATCATCTCT TCGTCGAAAT TAGATGTCCT
TGGCGTGACG CCTTTCGATG ACTGAAGTGA GTAGACATCA
GCGCCGTGAG TTGGTCCACC ACCGTAGCTG TTGGTGTACC CGTGTTTGGG
ACTAGCGGCC TTACTGGCAT TAAACATGGC GTAAAAATCA
GTCTGGTTGA AGCTCGATGC CCTCGGGGTC GGCTCTCGCG AGGATTGTAC
AGAGTAGATC CCAAGCTTCC CGGGTACCGC

AGC8 reverse compliment: [620bp]

GCGGTACCCG GGAAGCTTGG GATCTACTCT GTACAATCCT
CGCGAGAGCC GACCCCGAGG GCATCGAGCT TCAACCAGAC
TGATTTTTAC GCCATGTTTA ATGCCAGTAA GGCCGCTAGT CCCAAACACG
GGTACACCAA CAGCTACGGT GGTGGACCAA CTCACGGCGC
TGATGTCTAC TCACTTCAGT CATCGAAAGG CGTCACGCCA AGGACATCTA
ATTTCGACGA AGAGATGATG AAAAATATGG GAAAGAAGCA
GAGAGGAGCG AGGAGCATGA GTGGAGAGCT CTTCAATGGC
GGTCTTGCCC CGTCTTACCC TCCTCCTAAT CCGATGTTTT CCGGGTCGAC
AAGTAATACG GCTGTTAAGA AGAAAGACGC AACAACTGTT
ACAGCACCTC CTCCTGCTGC TGCTGCTGCT AATAATGGTC TGCCGAACAA
GGACCTTCAT ATGTTCGTAT GGAGCTCGAG TGCGTCGCCG
GTGTCGGAAG GGAACCTTAG ACACGCCGTT AACAGAGGTG
TCTCTTCTGA ATTTGGAGCT ATTGATACTT CTAAATCTTC TGGCGTGCCT
CAGAACGAAA GAGGTAGGGG ATCTTGGGAT CCAAGCTTCC
CGGGTACCGC

EXAMPLE 11

This example illustrates the amplicons produced during the amplification of STR locus **AGC 9** with multiplex cocktails comprising primer pairs SEQ ID NO: 21 and SEQ ID NO: 22.

Sequence for **AGC 9** locus:

GCGGTACCCGGGAAGCTTGGTACACTCTACATGGCTCAAATTCTCCC**GGTAA**
GTTGATACATTCCTTCCCAGCATGGAAAACAGAGTAGC**agcagcagcagcagcag**
cagcagcACGTCATATCAATCCAATTGCATTGTATTCTCCTTTAACTCATACAGCT
ATAGTTATGGCTGCCAACATATCTTCTCATCTCTTCCACTTAGCTTAATCAACT
CTCTTGGATACTAGGCAATTCGGTAACAGTTTACAAGTGTTAACCAGACGAC
AAAAAAAGAATTGTACACGTCCAGAATGGTGTCAGGGCCTACTAAAGGTTGA
ACCCAATTATTTTCTCAGGAATGGCTTTTGGCAAA**CAAGTAGCCTTTGGTCA**
CTGCCATTCTGAAGATCCCAAGCTTCCCGGGTACCGC

AGC9F: GGTAAGTTGA TACATTCCTT CCC [23bp]
AGC9R: CAAGTAGCCT TTGGTCACTG C [21bp]

AGC9F (rev. comp.): GGGAAGGAAT GTATCAACTT ACC [23bp]
AGC9R (rev. comp.): GCAGTGACCA AAGGCTACTT G [21bp]

AGC9 array: AGCAGCAGCA GCAGCAGCAG CAGC [24bp]
AGC9 motif: (AGCC)8

AGC9 locus: [411bp]
GCGGTACCCG GGAAGCTTGG TACACTCTAC ATGGCTCAAA
TTCTCCCGGT AAGTTGATAC ATTCCTTCCC AGCATGGAAA ACAGAGTAGC
CAGCAGCAGC AGCAGCAGCA GCAGCACGTC ATATCAATCC
AATTGCATTG TATTCTCCTT TAACTCATAC AGCTATAGTT ATGGCTGCCA
ACATATCTTC TCATCTCTTC CACTTAGCTT AATCAACTCT CTTGGATACT
AGGCAATTCG GTAACAGTTT ACAAGTGTTA ACCAGACGAC

AAAAAAAGAA TTGTACACGT CCAGAATGGT GTCAGGGCCT ACTAAAGGTT GAACCCAATT ATTTTCTCAG GAATGGCTTT TGGCAAACAA GTAGCCTTTG GTCACTGCCA TTCTGAAGAT CCCAAGCTTC CCGGGTACCG C

AGC9 reverse compliment: [411bp]

GCGGTACCCG GGAAGCTTGG GATCTTCAGA ATGGCAGTGA CCAAAGGCTA CTTGTTTGCC AAAAGCCATT CCTGAGAAAA TAATTGGGTT CAACCTTTAG TAGGCCCTGA CACCATTCTG GACGTGTACA ATTCTTTTTT TGTCGTCTGG TTAACACTTG TAAACTGTTA CCGAATTGCC TAGTATCCAA GAGAGTTGAT TAAGCTAAGT GGAAGAGATG AGAAGATATG TTGGCAGCCA TAACTATAGC TGTATGAGTT AAAGGAGAAT ACAATGCAAT TGGATTGATA TGACGTGCTG CTGCTGCTGC TGCTGCTGCT GGCTACTCTG TTTTCCATGC TGGGAAGGAA TGTATCAACT TACCGGGAGA ATTTGAGCCA TGTAGAGTGT ACCAAGCTTC CCGGGTACCG C

EXAMPLE 12

This example illustrates the amplicons produced during the amplification of STR locus **AGC 10** with multiplex cocktails comprising primer pairs SEQ ID NO: 23 and SEQ ID NO: 24.

Sequence for **AGC 10** locus:

GCGGTACCCGGGAAGCTT**GGATCAGCGGCAACAACAA**cagcaacaacaacatcagca**gcagcagcaacaacaacaacatcagcagcagcagcagcagcagcagcagcatcaacatcagcaacagcagca**acagcagcagcagcagcagcagcaacagcagcagcaacagcagcagcaacaacaccagcatcagcaacacca**gcagcagcaacaccagcatcagcagcaacatcagcagcagcagc**TTCAACCGTCACAACAATTGCA TCAGTTGTCTGTTCAGCAGCAGATTCCTAA**TGTTATGTCTGCTCTACCCAGT TTT**TCCTCTGGTACTCAGTCTCAGTCTCCATCGCTGCAGGCCATCCCTTCACA GTGCCAGCAGCCAAGCTTCCCGGGTACCGC

AGC10F: GGATCAGCGG CAACAACAA [19bp]

AGC10R: TGTTATGTCT GCTCTACCCA GTTTT [25bp]

AGC10F (rev. comp.): TTGTTGTTGC CGCTGATCC [19bp]
AGC10R (rev. comp.): AAAACTGGGT AGAGCAGACA TAACA [25bp]

AGC10 array: AGCAACAACA ACATCAGCAG CAGCAGCAAC AACAACAACA
TCAGCAGCAG CAGCAGCAGC AGCAGCAGCA GCATCAACAT
CAGCAACAGC AGCAACAGCA GCAGCAGCAG CAGCAGCAGC
AACAGCAGCA GCAACAGCAG CAGCAACAAC ACCAGCATCA
GCAACACCAG CAGCAGCAAC ACCAGCATCA GCAGCAACAT
CAGCAGCAGC AGC [213bp]

AGC10 motif: (AGC)1 + (AAC)3 + (ATC)1 + (AGC)4 + (AAC)4 + (ATC)1 + (AGC)10 + (ATC)1 + (AACATC)1 + (AGCAAC)1 + (AGC)2 + (AAC)1 + (AGC)8 + (AAC)1 + (AGC)3 + (AAC)1 + (AGC)3 + (AAC)2 + (ACC)1 + (AGC)1 + (ATC)1 + (AGC)1 + (AACACC)1+ (AGC)3 + (AACACC)1 + (AGC)3 + (AACACC)1 + (AGC)3 + (AACACC)1 + (AGCATC)1 + (AGC)2 + (AACATC)1 + (AGC)4

AGC10 locus: [408bp]
GCGGTACCCG GGAAGCTTGG ATCAGCGGCA ACAACAACAG
CAACAACAAC ATCAGCAGCA GCAGCAACAA CAACAACATC
AGCAGCAGCA GCAGCAGCAG CAGCAGCAGC ATCAACATCA
GCAACAGCAG CAACAGCAGC AGCAGCAGCA GCAGCAGCAA
CAGCAGCAGC AACAGCAGCA GCAACAACAC CAGCATCAGC
AACACCAGCA GCAGCAACAC CAGCATCAGC AGCAACATCA
GCAGCAGCAG CTTCAACCGT CACAACAATT GCATCAGTTG TCTGTTCAGC
AGCAGATTCC TAATGTTATG TCTGCTCTAC CCAGTTTTTC CTCTGGTACT
CAGTCTCAGT CTCCATCGCT GCAGGCCATC CCTTCACAGT GCCAGCAGCC
AAGCTTCCCG GGTACCGC

AGC10 reverse compliment: [408bp]
GCGGTACCCG GGAAGCTTGG CTGCTGGCAC TGTGAAGGGA
TGGCCTGCAG CGATGGAGAC TGAGACTGAG TACCAGAGGA
AAAACTGGGT AGAGCAGACA TAACATTAGG AATCTGCTGC

TGAACAGACA ACTGATGCAA TTGTTGTGAC GGTTGAAGCT
GCTGCTGCTG ATGTTGCTGC TGATGCTGGT GTTGCTGCTG CTGGTGTTGC
TGATGCTGGT GTTGTTGCTG CTGCTGTTGC TGCTGCTGTT GCTGCTGCTG
CTGCTGCTGC TGCTGTTGCT GCTGTTGCTG ATGTTGATGC TGCTGCTGCT
GCTGCTGCTG CTGCTGCTGA TGTTGTTGTT GTTGCTGCTG CTGCTGATGT
TGTTGTTGCT GTTGTTGTTG CCGCTGATCC AAGCTTCCCG GGTACCGC

EXAMPLE 13

This example illustrates the amplicons produced during the amplification of STR locus **ACT 1** with multiplex cocktails comprising primer pairs SEQ ID NO: 25 and SEQ ID NO: 26.

Sequence for **ACT 1** locus:

GCGGTACCCGGGAAGCTTGGGATCAAAAAACGAGAAGAATATTCATCATGA
AAAACTCTATAGAACTTTTATTATTCAAAGTAGGAAGGAACAAGGAAGAGGG
AAGAAAAAAAAAAGAAGGGGGCAGAGGGGGGCAATTTATGTTTGCCTTTTATG
CTATATATTTTAGTATCTAGAAGAACAAGAAAAAAAGACTATACTCCTAATA
TGAATATGGAACTAAAAAATT**GACTCAGCATATTAAAGCAGAAACT**TTGAA
ATAGACGAACCATGTTTTGGTTTACAACTGTGGTTTTTGTATTGACATCTAGT
TGTAAGGA**actactactactact**ACCTGTGCAAAAGGTGAACTCTCTACCATGAAAGT
AGTAATGGTTTTCAAGGGCCATTTAACTTGAACCACCATAGCTAGCAAAGGT
G**GTTTACATATTCCACTTGTTTGTGA**GCCACGCAAAGTGAGTTCCTATTAA
CCAGTTTTAAAACATATGTCATTTCCAAGATAGTTGAAAACCTCGGAAGCAG
CAGCATTACTGTTTTTCATAGCATTTCCAGGATTGTTGAAAACTTCAGCAGCA
GCAGCAGCAGCAACAGTATTACTGTTTTTTATAGCATCTCCATTTTGGTTCAC
AGTGAAATCCACAGTAAAGGAATTTAGACT

ACT1F: GACTCAGCAT ATTAAAGCAG AAACT [25bp]
ACT1R: GTTTACATAT TCCACTTGTT TGTGA [25bp]

ACT1F (rev. comp.): AGTTTCTGCT TTAATATGCT GAGTC [25bp]
ACT1R (rev. comp.): TCACAAACAA GTGGAATATG TAAAC [25bp]

ACT1 array: ACTACTACTA CTACT [15bp]
ACT1 motif: (ACT)5

ACT1 locus: [660bp]
GCGGTACCCG GGAAGCTTGG GATCAAAAAA CGAGAAGAAT
ATTCATCATG AAAAACTCTA TAGAACTTTT ATTATTCAAA GTAGGAAGGA
ACAAGGAAGA GGGAAGAAAA AAAAAGAAGG GGGCAGAGGG
GGGCAATTTA TGTTTGCCTT TTATGCTATA TATTTTAGTA TCTAGAAGAA
CAAGAAAAAA AGACTATACT CCTAATATGA ATATGGAACT
AAAAAATTGA CTCAGCATAT TAAAGCAGAA ACTTTGAAAT
AGACGAACCA TGTTTTGGTT TACAACTGTG GTTTTTGTAT TGACATCTAG
TTGTAAGGAA CTACTACTAC TACTACCTGT GCAAAAGGTG AACTCTCTAC
CATGAAAGTA GTAATGGTTT TCAAGGGCCA TTTAACTTGA
ACCACCATAG CTAGCAAAGG TGGTTTACAT ATTCCACTTG TTTGTGAGCC
ACGCAAAGTG AGTTCCTATT AACCAGTTTT AAAACATATG TCATTTCCAA
GATAGTTGAA AACCTCGGAA GCAGCAGCAT TACTGTTTTT CATAGCATTT
CCAGGATTGT TGAAAACTTC AGCAGCAGCA GCAGCAGCAA
CAGTATTACT GTTTTTTATA GCATCTCCAT TTTGGTTCAC AGTGAAATCC
ACAGTAAAGG AATTTAGACT

ACT1 reverse compliment: [660bp]
AGTCTAAATT CCTTTACTGT GGATTTCACT GTGAACCAAA ATGGAGATGC
TATAAAAAAC AGTAATACTG TTGCTGCTGC TGCTGCTGCT GAAGTTTTCA
ACAATCCTGG AAATGCTATG AAAAACAGTA ATGCTGCTGC
TTCCGAGGTT TTCAACTATC TTGGAAATGA CATATGTTTT AAAACTGGTT
AATAGGAACT CACTTTGCGT GGCTCACAAA CAAGTGGAAT
ATGTAAACCA CCTTTGCTAG CTATGGTGGT TCAAGTTAAA TGGCCCTTGA
AAACCATTAC TACTTTCATG GTAGAGAGTT CACCTTTTGC ACAGGTAGTA
GTAGTAGTAG TTCCTTACAA CTAGATGTCA ATACAAAAAC
CACAGTTGTA AACCAAAACA TGGTTCGTCT ATTTCAAAGT TTCTGCTTTA
ATATGCTGAG TCAATTTTTT AGTTCCATAT TCATATTAGG AGTATAGTCT

TTTTTTCTTG TTCTTCTAGA TACTAAAATA TATAGCATAA AAGGCAAACA TAAATTGCCC CCCTCTGCCC CCTTCTTTTT TTTTCTTCCC TCTTCCTTGT TCCTTCCTAC TTTGAATAAT AAAAGTTCTA TAGAGTTTTT CATGATGAAT ATTCTTCTCG TTTTTTGATC CCAAGCTTCC CGGGTACCGC

EXAMPLE 14

This example illustrates the amplicons produced during the amplification of STR locus **CCT 2** with multiplex cocktails comprising primer pairs SEQ ID NO: 27 and SEQ ID NO: 28.

Sequence for **CCT 2** locus:

GCGGTACCCGGGAAGCTTGGGATCGT**GCAGTGGATGTGTCGGGT**TCGAAA GTCTAT**cctcctcctcctcct**GCCGTTGGA
ATGGTGTGTTCGTCTCTGCCTGTTCAAAGAGCGACAATCAATGGTCTTAAAGG AGCACCTATCTGCCTGACTGGAAATCCAAGCTCCCTCCGATGAATGATTGTTT GTTCTTGCTTGATTACCGGAGGACCGACGCAGGAAGGCGTTGTCACTGCGAC TTGGTGCCTACTATGCTCTTCACGGAAAGGAGTGAAACGAGCAAGGAGAGAG TCAACCTTAATGTCAGTGATAATAGTAAAGGAAGAGACAGAATCTCATCTGC TTGGCTGGTCGACACAAGCAATGCCCAAAGAGCATTCTTTTCTATTTTCATGC TTCATAATGTATCCGCCGGATTGAAACAGTCTCT**TTTGTGCCTGACCTAATC CTCTA**GCTCTTTACTTGCCAGGAGAAGGCTCGCCAAGCTTCCCGGGTACCGC

CCT2F: GCAGTGGATG TGTCGGGT [18bp]
CCT2R: TTTGTGCCTG ACCTAATCCT CTA [23bp]

CCT2F (rev. comp.): ACCCGACACA TCCACTGC [18bp]
CCT2R (rev. comp.): TAGAGGATTA GGTCAGGCAC AAA [23bp]

CCT2 array: CCTCCTCCTC CTCCT [15bp]
CCT2 motif: (CCT)5

CCT 2 locus: [499bp]

GCGGTACCCG GGAAGCTTGG GATCGTGCAG TGGATGTGTC
GGGTTCGAAA GTCTATCCTC CTCCTCCTCC TGCCGTTGGA ATGGTGTGTT
CGTCTCTGCC TGTTCAAAGA GCGACAATCA ATGGTCTTAA
AGGAGCACCT ATCTGCCTGA CTGGAAATCC AAGCTCCCTC
CGATGAATGA TTGTTTGTTC TTGCTTGATT ACCGGAGGAC CGACGCAGGA
AGGCGTTGTC ACTGCGACTT GGTGCCTACT ATGCTCTTCA CGGAAAGGAG
TGAAACGAGC AAGGAGAGAG TCAACCTTAA TGTCAGTGAT
AATAGTAAAG GAAGAGACAG AATCTCATCT GCTTGGCTGG
TCGACACAAG CAATGCCCAA AGAGCATTCT TTTCTATTTT CATGCTTCAT
AATGTATCCG CCGGATTGAA ACAGTCTCTT TTGTGCCTGA CCTAATCCTC
TAGCTCTTTA CTTGCCAGGA GAAGGCTCGC CAAGCTTCCC GGGTACCGC

CCT 2 locus reverse compliment: [499bp]
GCGGTACCCG GGAAGCTTGG CGAGCCTTCT CCTGGCAAGT
AAAGAGCTAG AGGATTAGGT CAGGCACAAA AGAGACTGTT
TCAATCCGGC GGATACATTA TGAAGCATGA AAATAGAAAA
GAATGCTCTT TGGGCATTGC TTGTGTCGAC CAGCCAAGCA GATGAGATTC
TGTCTCTTCC TTTACTATTA TCACTGACAT TAAGGTTGAC TCTCTCCTTG
CTCGTTTCAC TCCTTTCCGT GAAGAGCATA GTAGGCACCA AGTCGCAGTG
ACAACGCCTT CCTGCGTCGG TCCTCCGGTA ATCAAGCAAG
AACAAACAAT CATTCATCGG AGGGAGCTTG GATTTCCAGT
CAGGCAGATA GGTGCTCCTT TAAGACCATT GATTGTCGCT CTTTGAACAG
GCAGAGACGA ACACACCATT CCAACGGCAG GAGGAGGAGG
AGGATAGACT TTCGAACCCG ACACATCCAC TGCACGATCC
CAAGCTTCCC GGGTACCGC

[0052] While certain of the preferred embodiments of the present invention have been described and specifically exemplified above, it is not intended that the invention be limited to such embodiments. Various modifications may be made thereto without departing from the scope and spirit of the present invention, as set forth in the following claims.

Table 1.
Collection of worldwide samples with representatives from all continents except Australia.

Continent

North America	# of Samples
U.S.A.	188
Canada	1
Mexico	7
Total North America	**196**

Central & South America	# of Samples
Colombia	3
Costa Rica	6
Jamaica	4
Total C & S America	**13**

Africa	# of Samples
Nigeria	1
South Africa	6
Sierra Leone	2
Uganda	2
Zimbabwe	2
Total Africa	**13**

Asia	# of Samples
Afghanistan	14
Cambodia	1
China	4
India	5
Japan	3
Korea	4
Kurdistan	2
Nepal	1
Pakistan	2
Russia	4
Thailand	1
Turkey	3
Uzbekistan	2
Total Asia	**46**

Table 1. Continued...

Europe	# of Samples
Czechoslovakia	1
France	3
Germany	4
Holland	2
Hungary	8
Italy	3
Poland	3
Romania	1
Spain	2
Total Europe	27

Total # Samples = 295

Table 2.
Attributes of eight microsatellite loci developed for *Cannabis sativa*. Values in the 'Amplicon Size Range (bp)' refer to results from fragment analyses of 295 *C. sativa* samples. 'Number of Alleles' reflects the number of alleles observed in this data set.

Locus Name Dye Label[a]	Primer Sequences	Repeat Motifs[b]	Amplicon Size Range (bp)	T_m (°C)	Number of Alleles	H_E
AAAG1 HEX	F: 5' GTCAGAAAGCGAAGACCTTTAGA 3' R: 5' GATGATGCCTGCCTGTCTTTAC 3'	(AAAG)6	103-135	59	16	0.684
AAAG5 NED	F: 5' GTCAATTAATGCTTATAGCCCATATGTTTTCTACTAC 3' R: 5' GCAACTTCAGGAATACTTTGTTTCTTCTTGTTCT 3'	(AAAG)5	188-200	59	4	0.625
AGC1 FAM	F: 5' GCAAAGAGTGTATCGAAACCTGTC 3' R: 5' GCCCACCACATCGTCTGTATTAGTAC 3'	(AGC)10	128-164	59	10	0.656
AGC6 HEX	F: 5' GAGACGTGGCATATGCGCTGTTCCTTCA 3' R: 5' GCCGATGGCCGTTCACTAATGGGTATGC 3'	(AGC)6	200 & 221	62	2	0.132
AGC8 NED & FAM	F: 5' GTTCCGACACCGGCGACGCACTC 3' R: 5' GTTCGACGAAGAGATGATGAAAAATATGGGAAAGAA 3'	(AGC)5	264-279	59	6	0.591
AGC9 HEX	F: 5' GGTAAGTTGATACATTCCTTCCC 3' R: 5' GCAGTGACCAAAGGCTACTTG 3'	(AGC)9	317-335	62	7	0.698
AGC10 NED	F: 5' GGATCAGCGGCAACAACAA 3' R: 5' GAAAACTGGGTAGAGCAGACATAACA 3'	(AGC)43	273-327	62	15	0.776
ACT1 FAM	F: 5' GACTCAGCATATTAAAGCAGAAACT 3' R: 5' GTCACAAACAAGTGGAATATGTAAAC 3'	(ACT)6	218-224	59	3	0.440

[a]HEX & FAM labeled primers were ordered from Integrative DNA Technologies; NED labeled primers were orcered from Perken Elmer

[b]Most repeat motifs are not perfect and appear to be complete

APPENDIX 1: Raw STR Data

Allelic scores, in base pairs, for all 295 samples genotyped across eight polymorphic loci. Samples where the same allelic size is listed twice are homozygous, whereas two different allelic sizes indicate a heterozygous state. Marker names are displayed across the top row of each page.

LEGEND

AFG	Afghanistan	UGA	Uganda
AK	Alaska, USA	UZB	Uzbekistan
AZ	Arizona, USA	WV	West Virginia, USA
CA	California, USA	ZIM	Zimbabwe
CAM	Cambodia		
CAN	Canada		
CHI	China		
COL	Colombia		
CoR	Costa Rica		
CT	Connecticut, USA		
CZE	Czechoslovakia		
FRA	France		
GER	Germany		
HA	Hawaii, USA		
HOL	Holland		
HUN	Hungary		
IND	India		
ITA	Italy		
JAM	Jamaica		
JAP	Japan		
KOR	Korea		
KURD	Kurdistan		
KY	Kentucky, USA		
MEX	Mexico		
NEP	Nepal		
NIG	Nigeria		
OR	Oregon, USA		
PAK	Pakistan		
POL	Poland		
ROM	Romania		
RUS	Russia		
SAF	South Africa		
SLe	Sierra Leone		
SPA	Spain		
THI	Thailand		
TN	Tennessee, USA		
TUR	Turkey		

Sample	AAAG1		ACT1		AGC8		AGC9		AGC1		AAAG5		AGC6		AGC10	
AFG177	127	127	221	221	264	270	326	326	152	152	192	192	200	200	309	321
AFG178	127	127	221	221	264	270	326	326	140	152	192	192	200	200	321	321
AFG181	103	117	221	221	270	270	326	326	140	152	196	196	200	200	294	303
AFG182	103	117	221	221	270	270	326	326	140	152	196	196	200	200	306	306
AFG217	117	117	218	221	264	264	326	326	152	164	192	192	200	200	303	309
AFG218	117	123	218	218	264	270	326	326	152	152	192	192	200	200	309	309
AFG223	117	127	218	221	270	270	332	332	131	131	188	196	200	200	309	309
AFG224	117	127	218	221	270	270	320	326	137	152	196	196	200	200	300	300
AFG225	127	127	221	221	267	267	320	323	140	152	188	192	200	200	300	309
AFG61	123	127	221	221	270	270	326	329	164	164	188	192	200	200	300	309
AFG62	117	127	218	221	270	270	326	326	131	131	192	192	200	221	300	300
AFG63	117	117	218	218	270	270	326	332	152	164	192	200	200	200	300	309
AFG64	127	127	218	218	270	270	326	326	152	164	192	192	200	200	309	324
AFG83	127	127	221	221	270	276	326	326	152	152	196	196	200	200	309	312
AK81	117	127	218	221	270	270	326	332	152	152	188	188	200	200	300	300
AK82	117	127	221	221	270	270	329	332	152	152	188	188	200	200	300	315
AZ100	117	127	218	218	270	276	323	332	131	152	196	200	200	221	309	315
AZ101	117	127	218	221	270	276	326	326	131	131	188	200	200	221	309	315
AZ102	117	123	221	221	264	270	323	332	131	152	188	200	200	200	315	315
AZ103	117	127	218	218	270	270	326	332	140	140	188	192	200	200	315	324
AZ104	117	117	218	221	270	276	323	332	131	152	192	200	200	200	309	315
AZ176	127	127	218	218	264	264	326	332	140	140	192	196	200	200	300	309
AZ97	117	123	221	221	264	270	323	332	131	152	188	200	200	200	315	315
AZ98	117	117	221	221	270	270	323	332	131	152	192	200	200	200	315	315
AZ99	127	127	218	221	264	276	326	326	152	152	192	196	221	221	309	309
CA121	117	127	221	221	264	276	323	329	137	152	188	192	200	200	315	321
CA122	117	117	221	221	264	270	326	329	137	152	192	192	200	200	312	321
CA123	117	117	221	221	264	270	323	323	137	152	192	192	200	200	309	315
CA124	121	123	221	221	264	264	323	326	152	152	192	192	200	200	306	309
CA125	123	127	218	221	264	270	326	329	152	152	192	192	200	200	306	306
CA126	127	127	218	221	264	270	326	332	131	152	192	196	200	200	312	315
CA127	127	127	218	221	264	270	326	332	131	152	192	196	200	200	312	315
CA128	117	117	224	224	270	270	326	326	140	152	188	188	200	200	300	300

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Sample	AAAG1		ACT1		AGC8		AGC9		AGC1		AAAG5		AGC6		AGC10	
CA129	117	117	221	221	270	270	326	326	140	152	188	188	200	200	300	300
CA130	113	123	221	221	264	264	326	329	152	152	192	192	200	200	309	315
CA131	113	123	221	221	264	264	326	329	152	152	188	192	200	200	309	315
CA132	127	127	218	218	270	279	326	326	152	152	188	192	200	200	309	309
CA133	113	117	218	221	264	264	329	329	152	152	192	192	200	200	315	324
CA134	123	127	221	221	270	270	326	326	152	152	192	192	200	200	309	309
CA135	127	127	218	221	264	264	326	326	152	152	192	200	200	200	309	309
CA136	117	117	221	221	264	270	326	326	152	152	188	192	200	200	309	312
CA137	117	117	221	221	270	270	323	329	152	152	192	192	200	200	309	321
CA138	117	117	221	221	270	270	326	326	152	152	188	192	200	200	309	309
CA139	117	117	221	221	264	270	326	326	152	152	192	192	200	200	309	309
CA140	117	117	218	221	264	270	323	326	146	152	192	192	200	200	312	315
CA141	117	117	221	221	264	270	323	326	152	152	192	192	200	200	300	309
CA142	117	117	221	221	264	270	323	326	152	152	192	192	200	200	300	309
CA143	117	117	218	221	264	264	326	329	152	152	188	188	200	200	309	315
CA144	117	117	218	221	264	264	326	329	152	152	188	188	200	200	309	315
CA145	117	121	221	221	264	270	323	326	137	152	192	192	200	200	309	309
CA146	117	121	221	221	264	270	323	326	137	152	192	192	200	200	309	309
CA147	117	117	218	221	264	264	326	329	152	152	188	188	200	200	309	315
CA148	117	117	218	221	270	270	326	326	140	152	192	196	200	200	300	309
CA149	127	127	221	221	264	264	326	326	152	152	188	192	200	200	300	318
CA150	117	117	218	221	264	264	320	329	152	152	192	192	200	200	288	309
CA72	117	127	221	221	270	270	323	326	152	152	188	192	200	200	300	300
CA73	117	127	221	221	270	270	326	326	152	152	188	188	200	200	309	324
CAM243	123	123	221	221	264	270	326	326	152	152	192	192	200	200	309	309
CAN231	117	117	218	218	264	270	320	329	146	146	192	192	200	200	297	306
CHI183	107	123	218	221	264	276	326	326	137	137	192	192	200	200	297	300
CHI184	117	119	218	218	270	270	326	326	137	137	192	192	200	200	297	321
CHI185	117	117	218	221	270	270	326	326	137	152	192	192	200	200	303	309
CHI201	111	123	221	221	270	279	320	326	146	152	196	200	200	200	297	303
COL67	117	117	221	221	264	279	323	326	152	152	188	188	200	200	309	309
COL68	117	117	221	221	264	273	326	329	131	164	188	192	200	221	303	315
COL69	117	117	221	221	279	279	323	326	152	152	188	192	200	200	309	309

Sample	AAAG1		ACT 1		AGC 8		AGC 9		AGC L		AAAG5		AGC 6		AGC 10	
CoR170	117	117	218	221	279	279	323	326	164	164	188	192	200	200	309	309
CoR171	117	117	221	221	270	273	323	323	164	164	188	188	200	200	309	309
CoR172	117	117	218	221	270	279	323	323	146	152	192	192	200	200	309	309
CoR173	117	117	218	218	264	279	323	326	146	152	188	192	200	200	309	309
CoR174	117	117	218	221	270	273	323	323	152	152	188	188	200	200	309	309
CoR175	117	117	218	218	270	270	323	326	152	152	188	188	200	200	309	309
CT 1	117	117	221	221	264	270	323	326	140	152	188	196	200	200	300	309
CT 2	117	117	221	221	264	270	323	326	140	152	188	196	200	200	300	309
CT 3	117	123	221	221	264	264	326	326	140	140	188	188	200	200	300	300
CT 4	117	117	218	221	264	270	326	332	152	152	188	188	200	200	300	309
CT 5	117	123	221	221	264	270	326	329	140	152	188	192	200	200	300	315
CT 6	117	123	221	221	264	270	326	329	140	152	188	192	200	200	300	315
CT 7	117	123	221	221	264	264	326	326	140	140	188	188	200	200	300	300
CT 8	117	117	221	221	264	264	326	326	140	152	188	188	200	200	300	300
CT 9	117	123	218	221	264	270	326	326	140	152	188	192	200	200	300	309
CT 10	117	127	221	221	264	270	326	326	152	152	188	192	200	221	300	300
CT 11	117	117	218	221	270	270	326	326	140	152	192	192	200	200	309	309
CT 12	117	117	221	221	264	279	332	332	140	152	188	188	200	200	309	309
CT 13	117	117	221	221	264	279	332	332	140	152	188	188	200	200	309	309
CT 14	123	123	221	221	270	270	326	332	140	152	188	188	200	200	309	309
CT 15	117	127	221	221	264	270	326	326	152	152	188	192	200	221	300	300
CT 16	117	123	221	221	264	264	326	326	140	140	188	188	200	200	300	300
CT 17	117	123	221	221	264	264	326	326	140	140	188	188	200	200	300	300
CT 18	117	127	221	221	264	270	326	326	152	152	188	192	200	221	300	300
CT 19	123	123	221	221	270	270	326	332	140	152	188	188	200	200	309	309
CT 20	117	127	221	221	264	270	326	326	152	152	188	192	200	221	300	300
CT 21	117	127	218	221	264	264	326	326	140	152	188	192	200	200	300	309
CT 22	117	127	221	221	264	273	326	326	131	152	188	196	200	200	300	321
CT 23	117	117	221	221	264	264	323	332	131	140	192	192	200	200	309	309
CT 24	117	117	221	221	270	270	326	326	152	152	188	188	200	200	309	309
CT 25	117	127	221	221	264	264	326	332	152	152	196	196	200	200	309	321
CT 26	117	127	221	221	264	270	323	326	140	140	188	196	200	200	309	309
CT 27	117	127	221	221	264	270	326	326	140	152	188	188	200	200	300	300

Sample	AAAG1		ACT1		AGC8		AGC9		AGC1		AAAG5		AGC6		AGC10	
CT28	117	127	221	221	264	270	323	326	140	140	188	196	200	200	309	309
CT29	127	127	221	221	264	270	326	326	131	131	188	200	200	200	321	321
CT30	117	117	221	221	264	270	326	332	152	152	188	192	200	200	309	309
CT31	117	117	221	221	270	270	323	326	140	152	188	196	200	200	300	309
CT32	117	117	221	221	264	270	323	326	140	152	188	188	200	200	309	309
CT33	117	123	221	221	270	273	326	326	152	152	188	188	200	200	300	300
CT34	117	117	221	221	270	270	323	326	140	152	188	196	200	200	300	309
CT35	117	127	218	221	264	270	326	326	152	152	188	192	200	200	309	309
CT36	117	127	221	221	264	273	326	326	131	152	188	196	200	200	300	321
CT37	127	127	221	221	264	270	323	326	131	140	192	196	200	200	309	321
CT38	117	117	221	221	276	279	323	332	140	152	188	192	200	200	309	309
CT39	117	117	221	221	276	276	332	332	152	152	188	188	200	200	309	309
CT40	117	127	221	221	264	273	326	326	131	152	188	196	200	200	300	321
CZE187	117	117	221	221	270	270	329	329	146	152	192	192	200	200	303	303
FRA189	103	117	218	218	264	270	332	332	134	146	192	192	200	200	294	303
FRA190	113	113	218	221	264	270	320	332	146	152	192	192	200	200	306	306
FRA193	117	125	221	221	264	270	332	332	134	146	192	192	200	200	318	336
GER188	117	117	218	221	264	264	332	332	146	152	188	192	200	200	312	312
GER195	117	117	218	218	264	270	329	332	128	146	192	192	200	200	303	303
GER240	115	117	221	221	264	279	326	329	146	146	192	192	200	200	294	309
GER91	103	117	221	221	279	279	320	320	146	152	192	192	200	200	321	321
HA209	117	117	221	221	264	279	326	329	152	152	188	192	200	200	309	315
HA210	117	127	221	221	264	279	326	326	152	152	188	192	200	200	309	315
HA211	117	127	221	221	270	270	326	332	131	164	188	192	200	200	309	324
HA77	117	117	218	221	264	270	326	329	137	164	188	192	200	200	297	300
HA78	117	127	221	221	264	264	329	329	152	164	188	196	200	200	315	315
HA79	117	123	221	221	264	264	326	326	152	164	188	188	200	200	300	300
HA80	117	127	221	221	264	264	326	329	152	152	188	188	200	200	300	300
HOL200	123	123	218	221	264	270	326	329	152	152	192	192	200	200	312	312
HOL230	117	117	221	221	264	273	323	326	140	152	188	188	200	200	300	309
HUN192	117	121	218	221	270	270	329	332	146	152	188	192	200	200	297	321
HUN198	117	117	221	221	270	279	326	332	146	152	192	192	200	200	303	318
HUN212	105	117	218	218	270	270	329	332	134	146	188	188	200	200	294	318

Sample	AAAG1		ACT1		AGC8		AGC9		AGC1		AAAG5		AGC6		AGC10	
HUN213	115	117	218	221	264	270	329	332	137	146	188	192	200	200	303	303
HUN70	117	117	218	218	270	270	332	332	146	146	188	192	200	200	315	321
HUN84	117	117	218	221	264	264	326	329	146	152	192	192	200	200	303	303
HUN87	117	121	218	221	264	270	326	329	137	152	188	188	200	200	273	303
HUN89	117	117	221	221	264	279	326	329	128	146	192	192	200	200	303	306
IND179	123	123	221	221	270	276	323	329	140	152	192	196	200	200	303	303
IND180	113	127	221	221	270	270	326	332	152	152	192	196	200	200	306	309
IND207	121	123	218	221	270	270	323	323	152	152	192	196	200	200	309	309
IND229	123	123	218	221	276	279	326	326	152	152	188	192	200	200	306	306
IND86	117	117	218	221	279	279	326	326	152	164	192	192	200	200	309	309
ITA191	117	117	218	221	270	270	317	329	146	152	192	192	200	200	297	306
ITA194	121	121	218	218	270	270	332	332	134	143	188	192	200	200	300	318
ITA88	103	117	218	218	264	270	320	329	146	152	192	192	200	200	306	306
JAM236	117	117	221	221	270	279	329	329	164	164	188	192	200	200	300	300
JAM237	117	117	221	221	264	270	329	329	164	164	188	188	200	200	309	309
JAM65	117	123	218	218	270	276	320	326	152	164	196	200	200	221	300	321
JAM66	127	127	218	221	270	270	326	329	152	164	188	200	200	200	309	309
JAP196	113	113	218	218	270	270	326	326	143	146	196	196	200	200	306	306
JAP241	109	123	221	221	270	270	320	320	128	128	192	200	200	200	306	306
JAP242	103	109	221	221	270	270	317	326	128	143	192	200	200	200	300	306
KOR186	109	113	218	221	270	270	320	326	128	146	192	200	200	200	321	321
KOR204	113	123	218	221	270	270	320	326	134	146	192	196	200	200	297	297
KOR248	113	117	221	221	270	270	326	329	128	128	192	196	200	200	297	306
KOR249	113	123	218	221	270	270	326	326	131	137	192	192	200	200	294	294
KURD214	119	119	221	221	264	264	326	326	128	152	192	192	200	200	294	294
KURD215	117	123	221	221	264	264	326	326	152	152	192	192	200	200	306	306
KY1	125	133	221	221	270	270	326	326	152	152	192	196	200	200	309	309
KY165	117	117	221	221	270	270	326	326	152	152	188	196	200	200	309	309
KY166	117	117	221	221	270	270	326	326	152	152	188	188	200	200	309	309
KY167	117	127	221	221	270	270	326	329	152	152	188	188	200	200	309	321
KY168	117	127	224	224	264	270	326	326	152	152	196	196	200	200	309	321
KY169	117	127	218	221	264	270	323	326	152	152	192	192	200	200	309	309
KY2	123	133	218	221	270	270	323	326	140	152	188	192	200	200	315	318

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Sample	AAAG1		ACT 1		AGC 8		AGC 9		AGC 1		AAAG5		AGC 6		AGC 10	
KY25	121	133	218	221	270	270	323	326	152	152	196	196	221	221	306	312
KY26	123	123	221	221	270	270	323	329	152	152	188	188	200	221	303	309
KY27	123	123	218	221	270	270	323	326	152	152	192	192	200	221	303	309
KY28	123	123	221	221	270	270	323	326	137	152	196	196	200	200	303	303
KY29	113	127	218	221	270	270	323	326	137	152	192	192	221	221	306	312
KY3	123	127	218	221	270	270	323	332	137	164	192	192	200	221	312	327
KY30	117	123	218	221	270	270	326	329	137	137	188	196	200	221	309	309
KY31	113	117	218	218	264	270	323	326	140	140	192	192	200	200	306	312
KY32	119	127	218	221	264	264	326	326	152	152	196	196	200	200	309	309
KY4	117	123	221	221	264	270	323	329	140	152	188	196	200	200	306	309
KY49	123	123	221	221	264	270	323	332	140	152	188	188	200	200	312	312
KY5	117	127	218	221	264	264	329	329	137	152	192	192	200	200	306	327
KY50	117	123	221	221	270	270	329	329	152	164	188	188	200	200	303	318
KY51	117	123	221	221	270	270	320	323	152	152	188	188	200	221	309	321
KY52	117	117	218	218	264	270	323	329	137	152	192	192	200	200	303	315
KY53	123	133	221	221	264	273	323	332	137	152	192	192	200	221	309	309
KY54	117	127	221	221	270	270	326	332	140	152	192	196	200	200	309	318
KY55	133	133	221	221	264	264	335	335	152	152	196	196	200	200	309	309
KY56	135	135	221	221	264	264	326	335	152	152	188	188	200	200	303	312
KY6	123	133	221	221	264	273	323	332	152	152	192	192	200	200	309	309
KY7	117	127	221	221	270	279	323	326	152	152	188	192	200	200	312	312
KY74	123	123	221	221	270	270	323	329	152	152	188	192	200	200	297	309
KY75	117	117	218	221	264	273	323	326	152	152	192	196	200	200	309	309
KY76	117	117	218	221	270	270	329	329	137	137	188	192	200	200	315	315
KY8	123	129	218	218	270	270	326	326	152	152	188	188	200	200	303	312
MEX233	117	121	218	218	264	264	320	329	137	152	192	192	200	200	318	318
MEX246	117	117	221	221	264	264	323	323	152	164	188	188	200	221	309	309
MEX57	117	117	218	221	264	270	323	323	131	152	188	192	200	200	309	309
MEX58	117	117	221	221	264	264	326	329	152	164	188	192	200	200	309	309
MEX59	113	117	218	218	270	270	329	332	152	164	192	192	221	221	288	315
MEX60	117	117	218	221	264	279	323	329	164	164	188	188	200	200	309	315
MEX85	117	117	218	221	264	270	323	329	152	164	188	188	200	221	309	315
NEP221	123	123	218	218	270	270	317	323	152	152	188	192	200	200	288	318

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Sample	AAAG1		ACT1		AGC8		AGC9		AGC1		AAAG5		AGC6		AGC10	
NIG222	123	123	218	218	264	270	323	326	134	155	192	192	200	200	309	309
OR93	117	117	221	221	264	270	326	332	140	152	188	188	200	200	300	309
OR94	117	117	221	221	264	270	326	332	140	152	188	188	200	200	300	309
PAK226	115	127	218	221	264	270	326	332	152	152	192	196	200	200	300	300
PAK227	117	127	218	218	264	264	326	326	152	152	188	196	200	200	300	300
POL216	117	117	218	221	270	270	332	332	146	146	188	188	200	200	300	318
POL228	121	123	221	221	264	270	332	332	134	152	192	192	200	200	297	297
POL71	121	121	221	221	270	270	332	332	131	152	192	192	200	200	303	303
ROM203	117	117	218	218	264	270	320	320	137	146	188	192	200	200	321	321
RUS197	117	121	218	218	270	270	329	332	146	146	192	192	200	200	300	303
RUS205	117	117	218	218	270	270	326	332	152	152	192	192	200	200	312	318
RUS206	117	117	218	218	270	270	326	332	137	140	192	192	200	200	300	312
RUS90	121	125	218	218	270	270	326	329	146	152	192	192	200	200	297	315
SAF208	115	115	221	221	276	276	326	335	128	152	192	192	200	200	309	309
SAF220	117	117	218	218	264	264	323	323	152	152	192	192	200	200	309	309
SAF247	123	123	221	221	264	264	323	323	152	155	192	192	200	200	309	309
SAF250	117	117	221	221	264	264	323	323	152	152	192	192	200	200	309	309
SAF251	117	123	218	218	264	264	323	326	152	152	192	192	200	200	309	309
SAF252	117	117	221	221	264	264	323	329	152	155	192	192	200	200	309	309
SLe234	123	123	218	218	270	270	323	326	152	155	192	192	200	200	309	309
SLe235	123	123	218	218	270	270	326	326	152	152	192	192	200	200	309	309
SPA202	119	123	221	221	270	270	326	329	128	143	192	192	200	200	306	306
SPA92	115	115	221	221	264	276	329	332	152	152	192	192	200	200	303	303
TH1232	123	123	221	221	270	270	326	326	152	152	192	192	200	200	300	309
TN10	117	123	221	221	270	270	320	323	152	152	192	192	200	221	303	309
TN105	123	123	218	221	270	279	323	323	128	128	188	192	200	200	309	309
TN106	123	123	218	221	270	279	323	323	128	128	188	192	200	200	309	309
TN107	127	127	221	221	264	264	326	326	152	152	188	188	200	200	303	303
TN108	117	117	221	221	264	270	326	329	152	152	188	188	200	200	303	312
TN109	117	117	221	221	264	276	323	329	128	146	188	192	200	200	309	309
TN11	117	127	221	221	264	276	326	332	152	164	192	192	200	221	309	309
TN110	117	127	218	221	264	270	326	332	152	164	192	200	200	200	309	315
TN111	117	127	218	221	264	270	326	332	152	164	192	200	200	200	309	315

Sample	AAAG1		ACT1		AGC8		AGC9		AGC1		AAAG5		AGC6		AGC10	
TN112	115	123	221	221	264	264	329	329	152	152	188	192	200	200	300	321
TN113	117	127	218	221	270	270	326	329	152	152	192	192	200	200	309	309
TN114	117	117	218	218	270	270	326	329	152	152	188	188	200	200	300	303
TN115	117	117	221	221	264	270	317	323	152	152	192	192	221	221	297	309
TN116	117	123	221	221	270	279	323	326	164	164	188	192	200	200	309	309
TN117	117	117	221	221	270	270	323	326	152	152	188	188	200	200	309	309
TN118	117	117	218	221	270	273	326	326	152	152	188	188	200	200	300	309
TN119	117	117	218	221	273	276	329	329	152	152	188	200	200	200	315	315
TN12	117	127	221	221	264	270	326	326	152	152	192	192	200	200	318	318
TN120	117	117	218	218	273	273	329	329	152	152	188	200	200	200	300	315
TN13	117	127	221	221	264	270	326	326	152	152	188	192	200	221	309	318
TN14	117	125	221	221	264	264	326	332	137	137	200	200	200	200	294	318
TN15	117	123	221	221	270	273	329	329	128	152	188	192	200	200	312	315
TN16	117	117	218	221	270	279	329	332	137	137	188	188	200	200	300	315
TN41	115	127	218	221	270	270	320	326	140	152	196	196	200	200	312	324
TN42	115	127	218	221	270	270	320	326	140	152	196	196	200	200	309	321
TN43	113	117	221	221	264	270	320	326	140	155	188	196	200	200	309	309
TN44	127	127	221	221	264	270	320	326	140	155	196	196	200	200	309	321
TN45	117	117	218	221	264	279	323	329	152	152	188	188	200	221	300	309
TN46	117	127	221	221	264	270	326	329	152	152	196	196	200	200	309	321
TN47	127	127	221	221	264	264	326	329	140	164	192	192	200	200	303	309
TN48	117	127	221	221	270	270	323	326	137	137	188	188	200	200	300	309
TN9	117	127	221	221	264	270	323	329	152	152	192	192	200	221	315	315
TUR199	113	117	218	221	264	264	326	326	134	152	192	192	200	200	309	312
TUR253	103	117	218	221	270	270	326	329	146	152	188	188	200	200	303	312
TUR254	115	117	218	218	270	270	326	329	152	152	188	192	200	200	303	309
UGA238	115	115	218	218	264	264	326	326	152	152	192	192	200	200	309	309
UGA239	115	115	218	218	264	264	326	326	152	152	192	192	200	200	309	309
UZB255	115	117	218	218	270	270	332	332	137	137	192	192	200	200	300	300
UZB256	123	123	218	221	264	270	323	326	137	152	192	196	200	200	306	306
WV151	113	117	221	221	270	270	323	326	152	164	192	196	221	221	300	309
WV152	117	117	218	221	270	270	323	323	137	152	192	192	200	200	318	318
WV153	127	127	218	221	264	264	326	329	152	152	188	192	200	221	315	321

Sample	AAAG1		ACT1		AGC8		AGC9		AGC1		AAAG5		AGC6		AGC10	
W V154	123	127	218	221	264	264	323	329	152	152	192	196	200	200	309	309
W V155	123	127	221	221	264	264	326	326	143	164	188	192	200	221	309	309
W V156	123	127	221	221	264	264	326	326	143	164	188	192	200	221	309	309
W V157	117	123	218	221	270	270	329	332	152	152	188	192	200	200	309	315
W V158	117	127	221	221	270	270	320	326	152	152	192	192	200	200	300	321
W V159	117	127	218	221	270	270	326	326	131	152	192	192	200	200	309	312
W V160	123	127	218	218	270	270	323	332	152	152	192	192	200	200	309	309
W V161	123	127	218	218	270	270	323	326	131	131	192	196	200	221	309	309
W V162	117	123	218	221	264	270	323	326	137	140	192	192	200	200	309	315
W V163	123	123	221	221	270	270	323	326	137	140	188	192	200	200	309	315
W V164	123	127	218	218	270	270	326	332	140	152	192	200	200	200	306	309
W V17	125	125	221	221	270	270	326	329	146	152	188	196	200	200	309	321
W V18	117	117	221	221	264	270	326	326	140	152	196	196	200	200	300	303
W V19	117	117	221	221	264	270	323	329	152	164	192	192	200	200	309	315
W V20	127	127	218	218	270	270	326	326	131	131	192	192	200	200	309	309
W V21	117	123	221	221	270	276	326	326	140	140	188	196	200	200	300	315
W V22	117	123	221	221	270	270	323	326	140	140	188	196	200	200	309	309
W V23	117	127	221	221	270	270	323	323	152	152	188	192	200	200	309	315
W V24	127	127	221	221	264	276	326	326	152	152	192	192	200	200	309	312
W V33	117	117	221	221	270	270	329	329	152	152	192	192	200	200	309	315
W V34	123	123	221	221	264	270	323	326	155	155	188	188	200	221	309	321
W V35	117	123	218	218	270	270	326	332	152	152	196	200	200	200	309	309
W V36	123	123	218	221	264	270	323	326	152	152	192	196	200	221	309	312
W V37	117	123	218	221	270	279	326	326	137	137	188	192	200	221	315	321
W V38	127	127	218	218	270	270	326	326	131	131	192	192	200	200	309	309
W V39	117	123	221	221	264	270	329	329	152	164	188	192	200	221	309	309
W V40	117	127	218	221	270	270	323	326	152	152	192	196	200	200	309	309
W V95	117	117	221	221	270	270	323	332	164	164	192	196	200	200	309	315
W V96	117	123	218	221	264	270	329	332	146	152	192	192	200	200	309	315
ZIM 244	117	123	218	218	264	270	323	326	152	155	192	192	200	200	309	309
ZIM 245	123	123	218	218	264	270	326	326	152	152	192	192	200	200	309	309

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REFERENCES

[1] G.S. Chopra, Man and marijuana, Int. J. Addiction, 4 (2) (1969) 215–247.

[2] R.E. Schultes, Man and marijuana, Nat. Hist. 82 (7) (1973) 58–63, 80, 82.

[3] R. Adams, Marihuana, Science 92 (1940) 115–119.

[4] E. Small, H.D. Beckstead, A. Chan, The evolution of cannabinoid phenotypes in *Cannabis*, Econ. Bot. 29 (1975) 219–232.

[5] W.A. Emboden, Jr., Ritual use of *Cannabis sativa* L.: a historical-ethnographic survey, in: P. T. Furst (Eds.), Flesh of the gods: the ritual use of hallucinogens, Praeger Publishers, NY and WA, 1972, pp. 214–235.

[6] E. Small, A. Cronquist, A practical and natural taxonomy for *Cannabis*, Taxon 25 (4) (1976) 405–435.

[7] R. Gillan, M.D. Cole, A. Linacre, J.W. Thorpe, N.D. Watson, Comparison of *Cannabis sativa* by random amplification of polymorphic DNA (RAPD) and HPLC of cannabinoids; a preliminary study, Sci. Justice 35 (3) (1995) 169–177.

[8] G. Siniscalco Gigliano, P. Caputo, S. Cozzolino, Ribosomal DNA analysis as a tool to identify specimens of *Cannabis sativa* L. of forensic interest, Sci. Justice 37 (3) (1997) 171–174.

[9] G. Siniscalco Gigliano, Identification of *Cannabis sativa* L. (*Cannabaceae*) using restriction profiles of the Internal Transcribed Spacer II (ITS2), Sci. Justice 38 (4) (1998) 225–230.

[10] G. Siniscalco Gigliano, Preliminary data on the usefulness of Internal Transcribed Spacer I (ITS1) sequence in *Cannabis sativa* L. identification, J. Forensic Sci. 44 (3) (1999) 475–477.

[11] M. Kohjyouma, I.J. Lee, O. Iida, K. Kurihara, K. Yamada, Y. Makino, S. Sekita, M. Satake, Intraspecific variation in *Cannabis sativa* L. based on intergenic spacer region of chloroplast DNA, Biol. Pharm. Bull. 23 (6) (2000) 727–730.

[12] S. Gilmore, R. Peakall, J. Robertson, Short tandem repeat (STR) DNA markers are hypervariable and informative in *Cannabis sativa*: implications for forensic investigations, Forensic Sci. Int. 131 (2003) 65–74.

[13] H.M. Hsieh, R.J. Hou, L.C. Tsai, C.S. Wei, S.W. Liu, L.H. Huang, Y.C. Kuo, A. Linacre, J.C.I. Lee, A highly polymorphic STR locus in *Cannabis sativa*, Forensic Sci. Int. 131 (2003) 53–58.

[14] D. Balding, Forensic applications of microsatellite markers, in D.B. Goldstein and C. Schlötterer (eds.), Microsatellites Evolution and Applications, (1999) Oxford University Press, New York, pp. 198-210.

[15] K. Weising, H. Nybom, K. Wolff, M. Wieland, DNA fingerprinting in plants and fungi, Boca Ration FL: CRC Press (1995) 51–53.

[16] S. H. Li, H. Yi-Jiun, J. L. Brown, Isolation of tetranucleotide microsatellites from the Mexican jay *Aphelocoma ulltramarina*, Mol. Ecol. 6 (1997) 499–501.

[17] T. Pearson, Polygyny and extra-pair paternity in a population of southwestern willoflycatchers (*Empidonax traillii extimus*), Northern Arizona University, M.S. thesis (2002)

[18] L. Cardle, L. Ramsay, D. Milbourne, M. Macaulay, D. Marshall, R. Waugh, Computational and experimental characterization of physically clustered simple sequence repeats in plants, Genetics 156 (2000) 847–854.

[19] S.D.E. Park, The Excel Microsatellite Toolkit; Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection, University of Dublin, Ph.D. thesis (2001).

[20] Anonymous, Microsat, the microsatellite distance program, Human population genetics laboratory, Department of genetics, Stanford, CA, USA, (2001).

[21] J. Flesenstein, PHYLIP (Phylogeny Inference Package) version 3.5c, Seattle, WA, USA, (1993).

[22] L. Excoffier, P.E. Smouse, J.M. Quattro, Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data, Genetics 131 (1992) 179–191.

[23] R. Peakall, P.E. Smouse, GenAlEx V5: Genetic Analysis in Excel. Population genetic software for teaching and research. Australian National University, Canberra, Australia (2001) www.anu.edu.au/BoZo/GenAlEx/

[24] M. Perez-Reyes, W.R. White, S.A. McDonald, R.E. Hicks, A.R. Jeffcoat, and C.E. Cook, The pharmacological effects of daily marijuana smoking in humans, Pharmacol. Biochem. Behav. 40 (1991) 691-694.

[25] C. Kimpton, D. Fisher, S. Watson, M. Adams, A. Urquhart, J. Lygo, P. Gill. Evaluation of an automated DNA profiling system employing multiplex amplification of four tetrameric STR loci, Int. J. Legal Med. 106 (1994) 302-311.

[26] D. Watts, Genotyping STR loci using an automated DNA sequencer: in P.J. Lincoln, J. Thompson (Eds.), Forensic DNA Profiling Protocols, Humana Press, Totowa, New Jersey, 1998, pp. 193-208.

[27] H. Miller Coyle, A.B. Harper, R.A. Bever, M.A. Sanger, J. Germano-Presby, K.N. Zinnamon, M.A. Menotti-Raymond, V.A. David, Non-human typing: methods and casework applications, Proceedings of the American Academy of Forensic Sciences 55th Annual Meeting, (2003) Feb. 17–22, Chicago, Illinois, USA. p. 20.

[28] H. Miller Coyle, C. Ladd, T. Palmbach, H.C. Lee, The green revolution: botanical contributions to forensics and drug enforcement, Croatian Med. J. 42 (3) (2001) 340–345.